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Design, Synthesis and Biological Evaluation of Novel Compounds with CNS-Activity Targeting Cannabinoid and Biogenic Amine Receptors

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Given the scope of this work, it would take a document of equal length to thank all of the family, friends, influences, and mentors throughout my life that have brought me to this point, so I must attempt to limit myself to the most relevant: first my parents, for their loving support as well as letting me find my own personal philosophies (as well as the associated the troubles and solutions); my high school chemistry teacher, Ms. Martin for providing my first academically humbling experience which largely influenced the early path of my undergraduate career; my thesis advisor Dr. Mark Trudell for allowing me to figure out things on my own, generate my own ideas, and ultimately treating me as a colleague; my thesis committee for generously donating their time and patience towards facilitating my success; my first academic employer Dr. Song Hong for teaching me about the tremendous work ethic and organizational skills required to be a successful researcher; and all of my wonderful friends – past, present, and future – who make my tiny existence worthwhile and so enjoyable.

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Abstract

This work seeks to contribute to the discipline of neuropharmacology by way of structure activity relationship from the standpoint of an organic chemist. More specifically, we sought to develop robust synthetic methodology able to efficiently produce an array of compounds for the purpose of systematic evaluation of their interaction with specific sights within the central nervous system (CNS) in order to better understand the mind and to develop drugs that may have beneficial effects on neurological function.

The focus of these studies has been toward the development of novel molecules, using a structure-activity relationship approach, that exhibit binding affinity at specific targets within the CNS. The merit of such studies is twofold: primarily, new compounds are produced that provide valuable scientific insight about their physiological targets, and secondarily, new synthetic methodologies that may arise in order to produce these compounds, thereby contributing to the whole of organic chemistry.

As a result of the research described herein, the development of one high affinity and several moderate affinity compounds at the cannabinoid receptor subtype 1 (CB1) has been accomplished. The research demonstrates that a diaryl ether molecular scaffold represents a successful motif in the cannabinoid pharmacophore. The production of the compounds in the SAR studies also introduced a novel general synthetic methodology for the synthesis of diaryl ethers around a phloroglucinol core.

A second project was initiated in order to explore the synthetic methods required to develop a general process for the synthesis of rigid aminobenzocyclobutane analogs of

known phenethylamines with activity at monoaminergic neurotransmitter sites. Using the synthetic approach devised here, four novel aminobenzocyclobutane isomeric analogs of a known pharmacologically active phenethylamine, (RS)-phenylpropan-amine were synthesized and are currently being evaluated for pharmacological potential.

Neuroscience, CNS-Active drugs, Cannabinoids, CB1 Receptor, Biogenic Amines, Serotonin, Dopamine, Norepinephrine, Drug Abuse, Addiction Pharmacotherapy, Organic Synthesis, Drug design

1

Introduction

1.1 CNS-active drugs, organic chemistry and the study of the mind

This introduction begins with a broad historical overview citing some early milestones in CNS-drug development and their relation to the interwoven collaborative efforts between early chemists and pharmacologists. In doing so, our motivation in completing this work of chemistry and pharmacology shall hopefully become partially revealed. As this introduction progresses, the focus will narrow accordingly and ultimately lead into the tiny sliver of new information contributed by the subsequent chapters. The discussion begins with a broad overview of the first synthetic CNS-active drugs and the interesting early history of these compounds. The development of the first synthetic CNS-active drugs was paralleled by the emerging support of the receptor theory of drugs, which will subsequently be reviewed. Receptor theory leads to a review of the pharmacodynamic techniques used to access the activity of drugs followed by an analysis of the specific receptor family which nearly all known CNS active drugs affect, the G-protein coupled receptors. Finally, two specific GPCR sites in the CNS and their corresponding ligands—the cannabinoids and monoamine neurotransmitters—will be discussed in some detail. The goal of this introduction is to funnel the reader into the ultimate rationale behind

completing the work described in this dissertation, along with providing suitable background information to support the subsequent chapters.

1.1.1 Why develop compounds that affect the CNS?

A CNS-active drug may be broadly defined as a compound able to cross the blood-brain barrier and cause changes in perception, mood, consciousness, cognition, and behavior. One of the simplest methods for studying the underlying mechanisms and functioning of the brain is by observing physiological responses elicited by external chemical influences, or drugs that function as "molecular probes." A wealth of knowledge about the body and brain has been acquired in the last century by studying the correlation between the structural features of chemical species and how they interact with the CNS, both at the cellular level and universally. In doing so, we have begun to develop at least a partial understanding of the endogenous mechanisms by which the CNS functions under normal physiological conditions. In turn, we are provided with valuable information for understanding the pathophysiology of the mind when affected by disease; subsequently, we are provided with potential treatments for easing the suffering associated with these conditions. Studying the underlying mechanisms of the CNS by way of "chemical probes" contributes valuable information towards understanding one of the greatest puzzles in science: the human brain.

1.1.2 A Brief History of the First CNS-Active Drugs

Humans have been utilizing plants, herbs and other naturally occurring substances to treat illness and manipulate physiological functions for thousands of years. However, it was the almost concurrent advent of organic chemistry and modern pharmacology in the late nineteenth century that has provided a systematic means by which to study and understand how exogenous organic compounds affect the mind and body. The roots of this revolution can be traced back to approximately 1804 with the extraction and isolation of the principle active component of the opium poppy, morphine, by Austrian pharmacist Friedrich Wilhelm Sertürner.[1] Using the principles of acid/base extraction, a technique that is still ubiquitous in any modern organic chemistry laboratory, morphine became the first isolated active ingredient from an herb or other plant. Application of the scientific method to isolating and purifying natural products, obtaining new compounds by chemical synthesis, and observing their physiological attributes have since become central themes in the disciplines of organic chemistry and pharmacology.

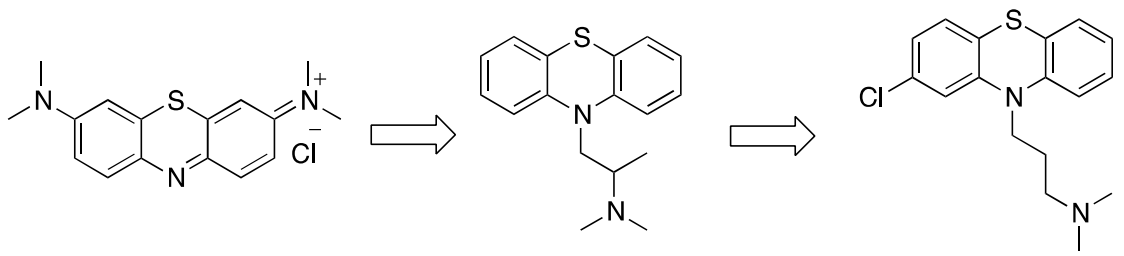
The development of early CNS-active drugs owes it beginnings in part to antibiotics and Fleming's discovery of penicillin in the 1920's as well as World War II for increasing the demand for the substance, as these factors provided the basis for the pharmaceutical industry that exists today. Once an effective antibiotic was identified in a naturally occurring source, organic chemists were employed to develop variations of the structure by chemical modification in order to produce patentable compounds with improved safety and efficacy that could be produced on large scale. The structure-activity relationship

methodology became a universal approach for the development of new drugs, including CNS-medications.[2]

A series of chance discoveries between the 1930's and 1960's helped set the stage for the development of many drugs that helped scientists understand and treat disorders of the mind. A classic example of one of these serendipitous discoveries was the development of chlorpromazine (**1.3**), the first effective treatment for schizophrenia, from a commonly used industrial dye.[3] The dye, methylene blue (**1.1**), introduced a new class of molecules called phenothiazines in the late 1800's, which had interesting properties aside from turning things blue. Specifically, methylene blue demonstrated antimalarial properties when ingested.[4] Efforts in the early 1940's to synthetically modify phenothiazines to produce more effective antimalarial agents largely failed, but the researchers noticed that some of the compounds synthesized had antiemetic and sedative properties in individuals on which they were tested (many years later, these drugs would collectively become known as antihistamines).[4] One of the most effective compounds at inducing sedation was called promethazine (**1.2**). In an attempt to further exploit the favorable sedative properties of promethazine and produce something useful for inducing anesthesia before surgery, Paul Charpentier working for Rhône-Poulenc (a French pharmaceutical company) synthesized a series of related compounds including one to become known as chlorpromazine (**1.3**) in 1950. In a clinical setting, physician Henri Laborit observed that chlorpromazine produced, "a tendency to sleep and disinterest in the surroundings."[5,6] In the same report, Laborit mentioned that this calming property might potentially be useful for treating individuals afflicted with schizophrenia, and subsequently, chlorpromazine was made available to professionals in psychiatry. The therapeutic potential of

chlorpromazine was immediately realized and within three years (1953-1955), chlorpromazine was being used around the world as a treatment for schizophrenia.[7]

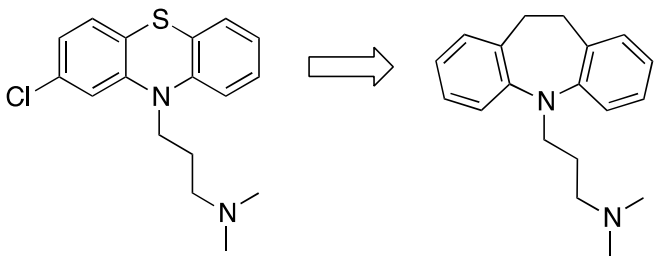
Scheme 1.1 From blue dye to the first pharmacological treatments for schizophrenia



1.1 **1.2** **1.3**

The drug imipramine (**1.4**) came about as a result of further modification of the chlorpromazine scaffold to produce a new class of compounds based on an iminodibenzyl system. Unlike chlorpromazine, it was found to not be particularly useful as an antipsychotic drug and actually made the symptoms of agitated psychotic patients slightly worse, with the exception being patients who exhibited symptoms of depression. To the researchers' surprise, it was noted that depressed individuals became more animated upon treatment with imipramine. These observations ultimately led to a new class of drugs known as tricyclic anti-depressants (TCAs), which are still prescribed today.[7]

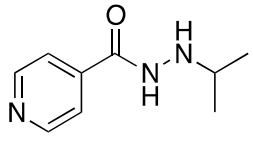
Scheme 1.2. From chlorpromazine to the first tricyclic antidepressants



1.3 **1.4**

Around the same time as the development of imipramine, research was underway to develop hydrazine-derived compounds that could potentially treat tuberculosis. While its use as a treatment for tuberculosis largely failed, it was discovered that patients who were administered the drug ipronazid (**1.5**) became "inappropriately happy."[8] Along with imipramine, ipronazid became one of the first pharmacological treatments for depression. It would eventually be discovered that ipronazid acted by inhibiting the enzyme monoamine oxidase and years later would lead to the discovery of an entire class of drugs that behaved similarly; these drugs are collectively known as monoamine oxidase inhibitors or MAOIs.[9]

Figure 1.1. Ipronazid, the first MAOI antidepressant

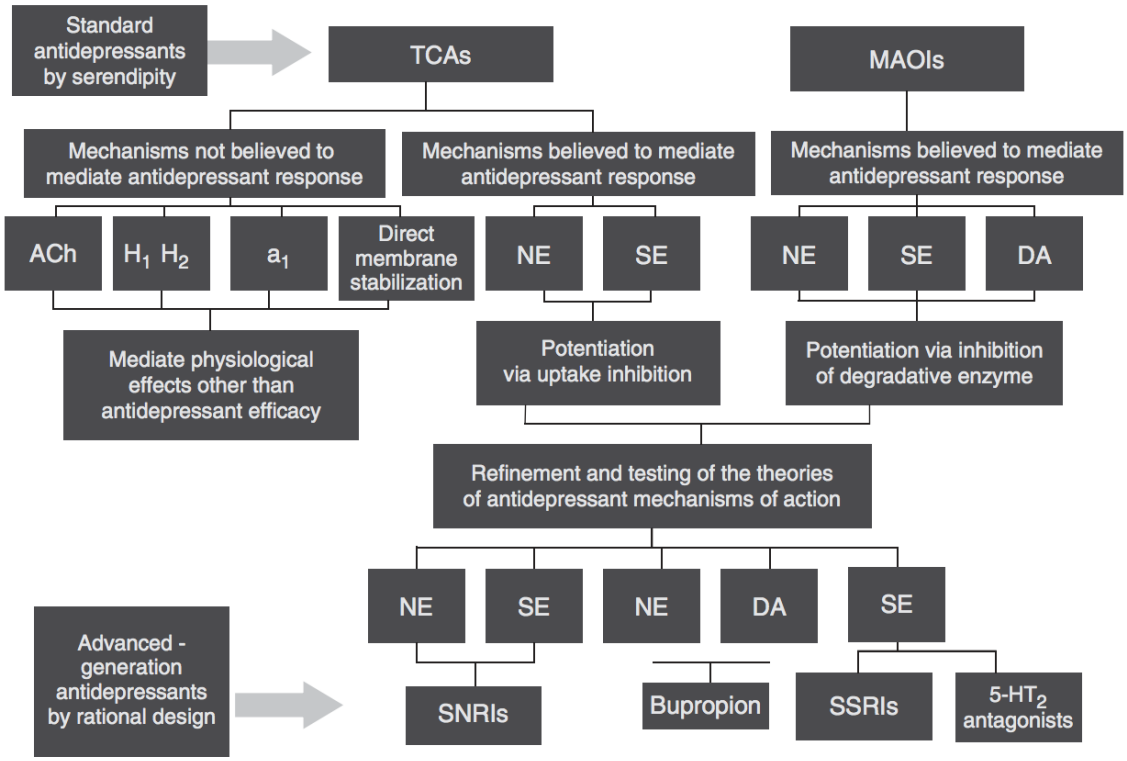


1.5

In addition to chlorpromazine, iproniazid, and imipramine, CNS-active drugs such as lithium and the barbiturates also gained popularity during the first half of the twentieth

century. These compounds helped to validate the pioneering work of researchers such as Otto Loewi, who in 1921, demonstrated for the first time that neurons were able to communicate by chemical messengers, later dubbed neurotransmitters. In addition to contributing to our basic understanding of the mind, these compounds served as probes into brain function and fundamentally changed the way medical professionals viewed psychiatric syndromes, providing a chemical basis by which psychiatric disorders could be understood.[2]

Figure 1.2. Timeline of CNS drug development[2]



With the solidification of receptor occupancy theory in the 1950's (discussed below) and experimental evidence describing the existence of neurotransmitters, a new era of

drug design began to emerge where novel drugs could be discovered by a means other than luck, serendipity, and ethically questionable human clinical trials. The concept of a receptor provided a target at which chemists could aim and a theoretical basis upon which compounds could be designed. As illustrated by **Figure 1.2**, the chance discoveries between the 1930's and 1960's that introduced tricyclic antidepressants and monoamine oxidase inhibitors set the stage for scientists to devise new theories about how these compounds affected the brain. As a result, these compounds helped to solidify many concepts underlying CNS functioning via neurotransmitters and the mechanisms by which they mediate specific processes in the body.

A comprehensive review of the subsequent advances in CNS-active drug design that occurred from the 1960's to present day and contributed to our current understanding of the mind would be a daunting task well beyond the scope of this work. The remainder of this introduction shall focus on the tenets of rational drug design and its founding principles followed by a discussion of the current state of the art in two frontiers in the study of the mind: the biogenic amines and the cannabinoids. This will conveniently transition into the research that is the subject of this dissertation in the subsequent chapters.

1.2 Early History of Pharmacology and Receptor Theory

During the first half of the twentieth century, where the previously mentioned compounds and many others were being discovered by luck, serendipity, and basic scientific experimentation, the actions of these compounds were only explained vaguely

and with reference to affinities to certain organs and tissues or even extraordinary chemical powers.[10] In an effort to provide a more rigorous and formal explanation regarding the actions of drugs, the discipline of pharmacology developed alongside these milestone drug discoveries during this time period.

The emergence of drug-receptor theory came about as a result of several conclusions published by Cambridge physiologist John Newport Langley (1852-1925) and the Berlin immunologist Paul Ehrlich (1854-1925). In 1905, following nearly 30 years of observations, Langley concluded that the naturally derived toxins nicotine and curare act on "receptive substances on muscle cells."[11] Meanwhile, based partially on his observations of the interactions of dyes with cells (histological staining), Ehrlich proposed the existence of "chemoreceptors" for drugs in 1907.[12] However, until about 1930, receptor theory was otherwise not well supported theoretically or experimentally and therefore was not widely accepted, and several alternative theories had been proposed during this time.[13]

Quantitative analysis of drug action on cells by British pharmacologist Alfred Joseph Clark (1885-1941) helped garner significant support for the concept of drug receptors in the early 1930's. In his 1933 publication, *The Mode of Action of Drugs on Cells*, Clark sought to apply concepts of physical chemistry to the interactions between drugs and cells.[14] He reasoned that from calculations describing the relatively small size of molecules such as adrenaline and acetylcholine at sparse physiological concentrations compared to the relatively large surface area of the heart cells that they affected, they were likely to "…exert their action by uniting with certain specific receptors in or on heart cells," and furthermore, that these receptors formed only an insignificant proportion of the total surface of cells. His approach recognized that the Law of Mass Action probably governed the rate at which

drugs reversibly bound to receptors in cells as an equilibrium process. He noted that the relation between drug concentration and the elicited biological effect followed a simple hyperbolic function in the same way that a gas reversibly adsorbs onto a metal surface at different concentrations, which was a principle already well studied in physical chemistry. Clark concluded that the equilibrium between a drug present in excess that reacts with a finite number of cell receptors to form an easily dissociable compound would produce the same hyperbolic curve as gas adsorption on a metal surface. His work would collectively become known as receptor occupancy theory. With R. P. Stephenson's introduction of the concept of efficacy (the ability to induce an effect after binding) to receptor occupancy theory in 1956, the basis of modern pharmacodynamic analysis had been established.[14,15]

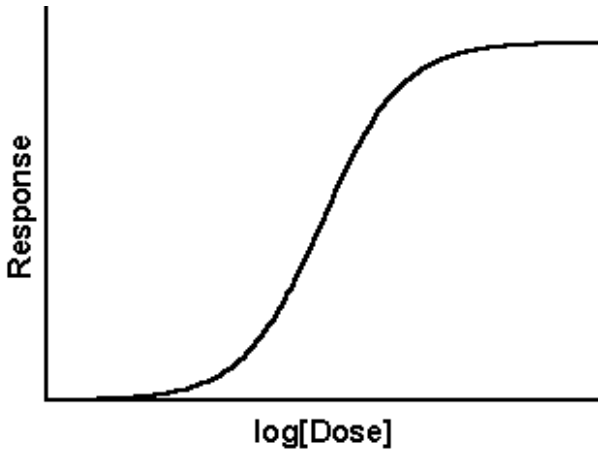
Further advances in providing the experimental evidence to support receptor occupancy theory relied heavily on the collaboration between organic chemists and pharmacologists, as the ability to study the pharmacological properties of receptors relied, by definition, on the availability of compounds able to selectively stimulate or antagonize the effects elicited by the receptors. Furthermore, perhaps the greatest contribution that organic chemistry has made to the study of pharmacology and the establishment of receptor theory was the synthesis of the first radioactively labeled drugs that provided the necessary probes able to demonstrate the existence of and visualize the receptors within the plasma membrane in the 1960-70's.[16,17] In the sections that follow, the focus will narrow upon the experimental and theoretical elements necessary for understanding and quantifying the actions of drugs on receptors, and in turn, how we may strategically design drugs targeting specific receptors based on these principles.

1.2.1 Binding affinity

The mathematics introduced by the Law of Mass Action to describe the concentration-response data provided a strong theoretical basis to describe the actions of drugs. A macroscopic static representation of this concept is shown in **Figure 1.3**, where a free ligand (L) at some concentration may bind reversibly with a receptor (R) forming receptor ligand complex (RL). Experimentally, if one quantified and plotted the cellular or physiological response elicited by varying concentrations of ligand (L), a hyperbolic curve would result. Conventionally, the logarithm of concentration is used for these plots, producing the sigmoidal dose-response curve shown in **Figure 1.4**. If the binding affinity of ligand (L) is defined by the chemical forces that cause the drug to associate with the receptor, then this property can be viewed in terms of the equilibrium of bound and unbound state to the receptor at given concentrations. The term K_d is called the dissociation constant and its value, with units of concentration, reflects the affinity of the ligand to the receptor, described by the relation $K_d = [R][L] / [RL]$. The value K_d is defined as the concentration of ligand that occupies 50% of the receptors at equilibrium, *i.e.* when $[R] = [RL]$; $K_d = [L]$, and therefore, a smaller concentration of K_d reflects a higher affinity of ligand L for receptor R.

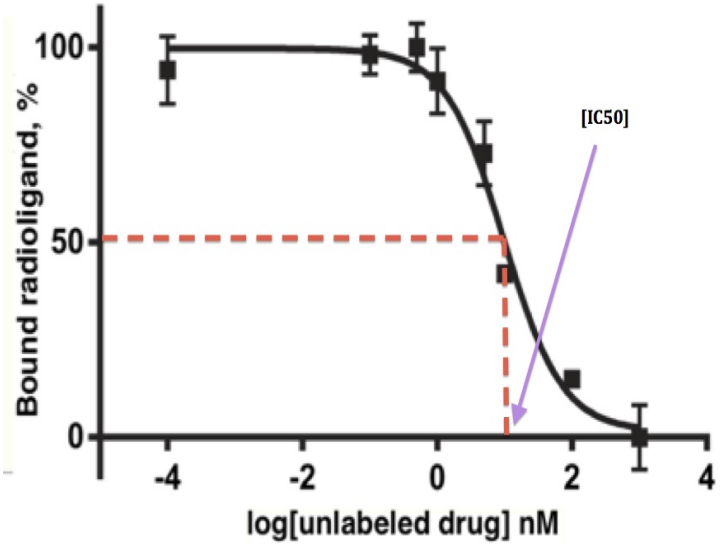
Figure 1.3. Schematic of ligand receptor binding

Figure 1.4. Sigmoidal dose-response curve of ligand receptor action



For the purpose of this dissertation, the binding affinities of novel compounds were determined by an experimental technique called a radioligand competitive binding assay. This procedure allowed us to determine the binding affinity of unknown compounds by measuring their ability to displace a radioactive tritium-labeled compound with a known dissociation constant at a specific receptor site of interest. Experimentally, this assay was conducted by adding a range of concentrations of the unknown compound to a radioligand bound to receptors at a fixed concentration. The output of this type of experiment was ideally a sigmoidal curve of the form shown in **Figure 1.5**, where the vertical axis represented the amount of radioligand bound to receptors and the horizontal axis represented increasing concentrations of unknown compound. From the curve, the IC_{50} value of the novel compound may be determined, which is the concentration of unlabeled drug that displaced 50% of the radioligand. From the IC_{50} value and known data about the radioligand, the Cheng-Prusoff equation (**Figure 1.5**) was used to produce the K_i value, or inhibition constant, for the drug, which represents an absolute value defined as the concentration of drug that would occupy 50% of receptors in the absence of radioligand. The experimentally determined K_i value should therefore be equal to the K_d value for the same compound, meaning the lower the value of this concentration, the higher the affinity of the compound for the receptor.

Figure 1.5. (Left) Characteristic sigmoidal curve produced by a radioligand competition binding assay. (Right) The Cheng-Prusoff equation: IC_{50} is measured from unknown ligand; [L] and K_d represent radioligand concentration and dissociation constant, respectively.

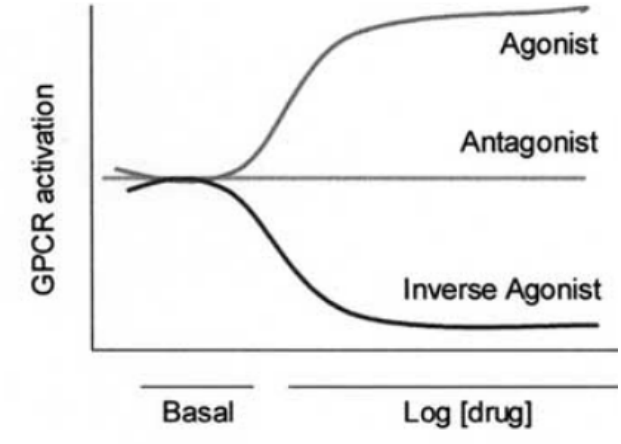


$$K_I = \frac{IC_{50}}{1 + [L]/K_D}$$

1.2.2 Drug efficacy

The word efficacy, also known as intrinsic activity, is a somewhat relative term that describes the degree by which a drug-receptor complex elicits a functional response. That is, if compound A produces a diminished physiological response compared to compound B at the same receptor site, compound A is said to have lower efficacy than compound B. The terms agonist, partial agonist, neutral antagonist, and inverse agonist are used to describe drug efficacy. A graphical representation in terms of dose-response relationship is displayed in **Figure 1.6**. An expanded discussion regarding the theory of efficacy is outlined below in **Section 1.3** with specific regard to the cellular response elicited by G-protein coupled receptors.

Figure 1.6. Comparison of drugs with the same binding affinity but different efficacies



1.2.3 G-Protein coupled receptors

The elucidation of GPCRs began around the early 1960's. The legacy left by the early pioneers of pharmacology such as Langley, Ehrlich, Stephenson, and Clark to the field of GPCR study was the concept of affinity and efficacy and the quantitative methods by which these properties could be understood. This theory combined with a diverse array of compounds able to differentially interact with the receptors has provided many of the experimental tools necessary in constructing our understanding of these receptors. Today, approximately 40-50% of drugs that are used clinically in humans and nearly 60-70% of those in development today target a G-Protein coupled receptor.[18–21]

The G-protein coupled receptor (GPCR) family represents the most diverse and versatile group of cell surface receptors known. These proteins serve as sensors capable of transducing an extracellular stimulus across the cell membrane barrier to activate intracellular signaling cascades. GPCRs consist of lipid bilayer spanning proteins and are characterized by seven transmembrane α-helices, known as the transmembrane domain. A

schematic representation of a GPCR is illustrated in **Figure 1.7**. The non-transmembrane regions of the receptor exist in aqueous intra- and extracellular environments and typically take the form of random, helical, or β-sheet conformations. The C-terminal segment connects to transmembrane-7 in the intracellular space and the N-terminal segment connects to transmembrane-1 in the extracellular space. Transmembrane helices are connected by three extracellular loops (Exoloops 1-3) and three intracellular loops (Cytoloops 1-3) in the cytosolic space.[22]

Figure 1.7. Cartoon illustration of a G-protein coupled receptor viewed from within cell membrane[23]

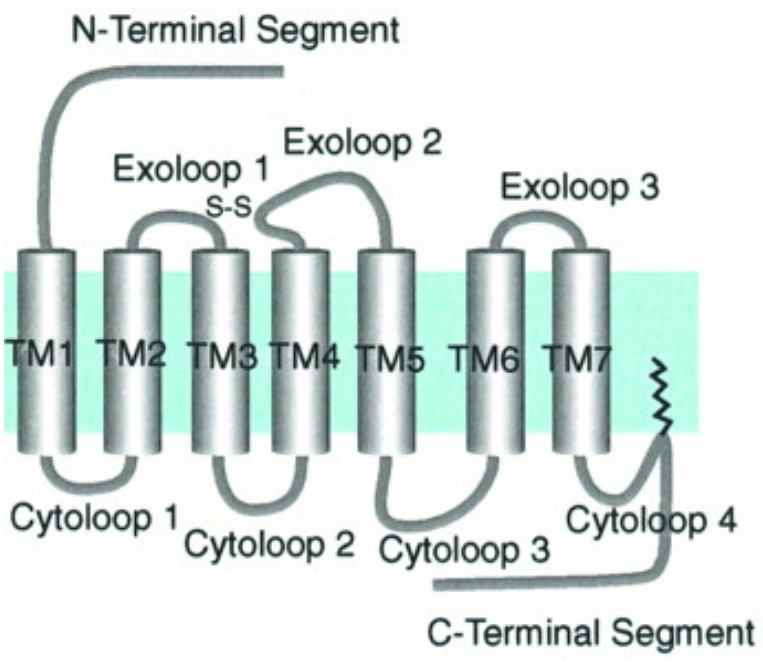
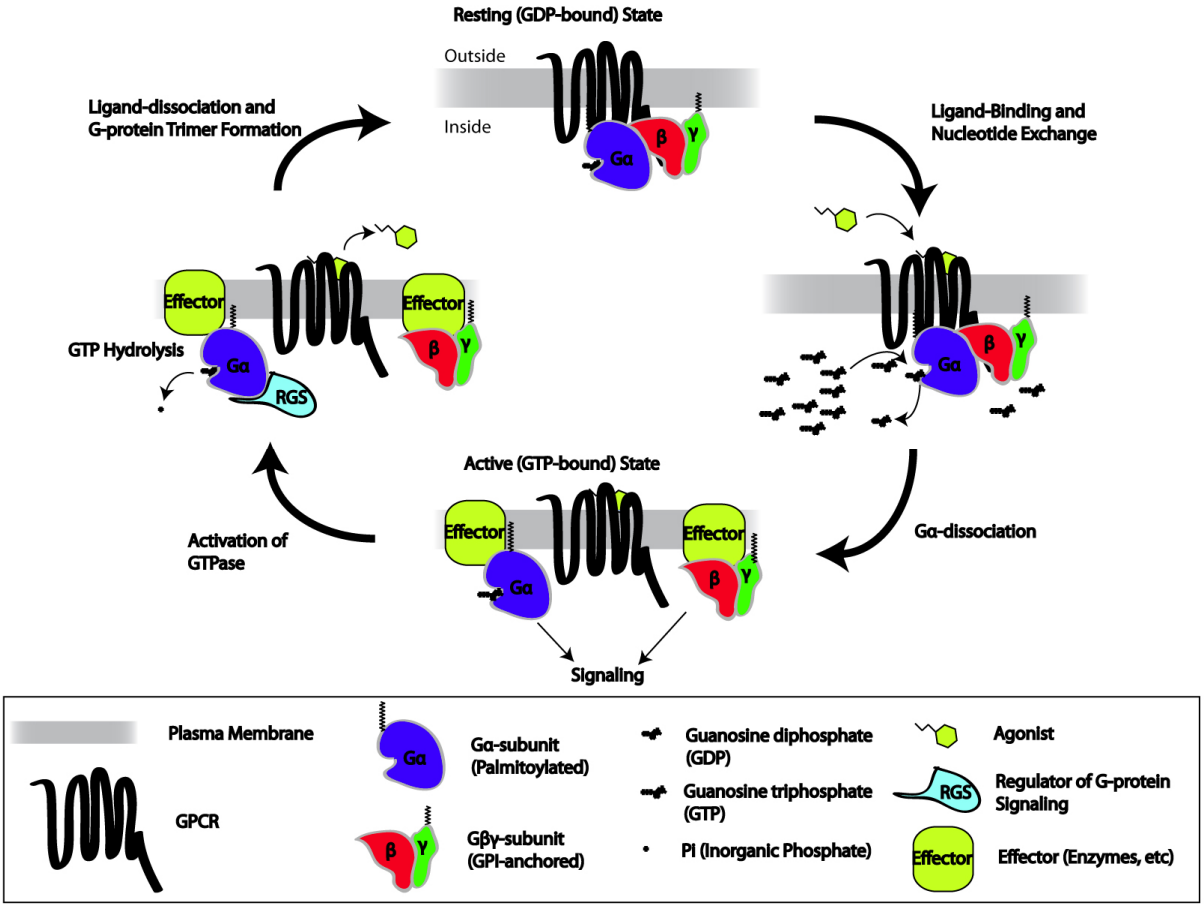


Figure 1.8 contains a cartoon illustration of the GPCR activation mechanism. The intracellular signaling cascades are primarily mediated by heterotrimeric GTP-binding proteins (G-proteins), which are activated or inactivated by conformational changes within

the receptor. When a G-protein is activated by a receptor conformational change, it exchanges GDP for GTP and the α subunit dissociates from the β-γ subunits, which stay associated with the intracellular membrane. The subunits then become free to activate various signaling cascades via enzymatic action—typically, cAMP, diacylglycerol, and/or phosphatidylinositol signal pathways.[22]

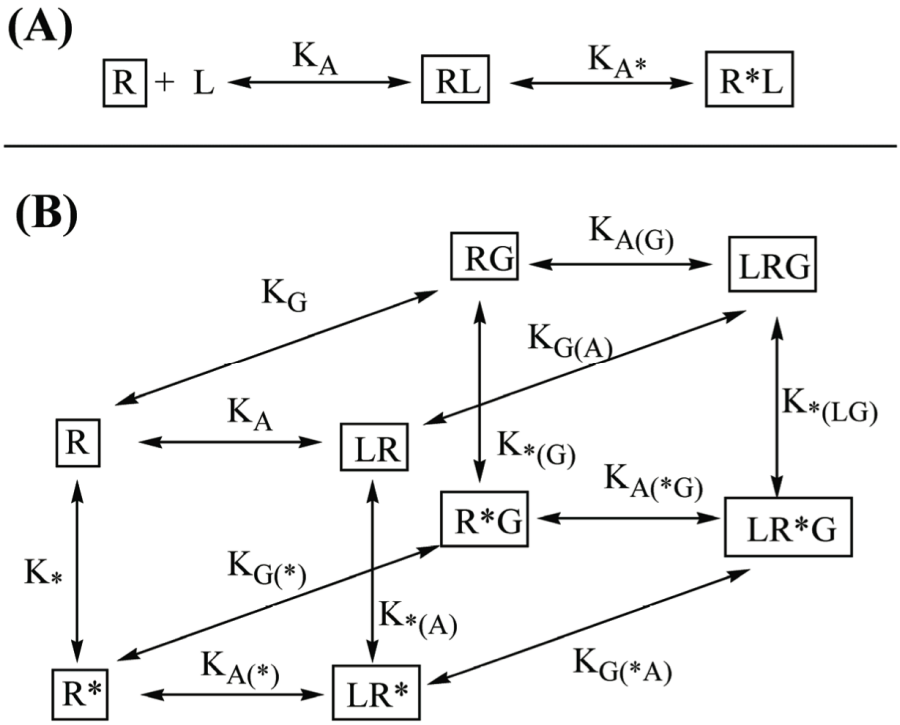
Figure 1.8. Cartoon illustration of GPCR activation cycle[24]



While it is sufficient in some cases to explain binding affinity and efficacy, the model of GPCR activation as a linear sequence of events in which the receptor acts as a "molecular

switch" when a ligand (L) binds is simplistic and not an entirely accurate depiction of the dynamics of the process (**Figure 1.9A**). The process is more thoroughly described by the dynamic nature of the conformational state of the receptor protein. An improved model called the cubic ternary complex shown in **Figure 1.9B** demonstrates a more refined approach to understanding GPCR functioning and considers the equilibrium of multiple combinations of ligand, receptor, and effector proteins, as well as taking into account that receptors exist in a dynamic equilibrium of conformational states.[25]

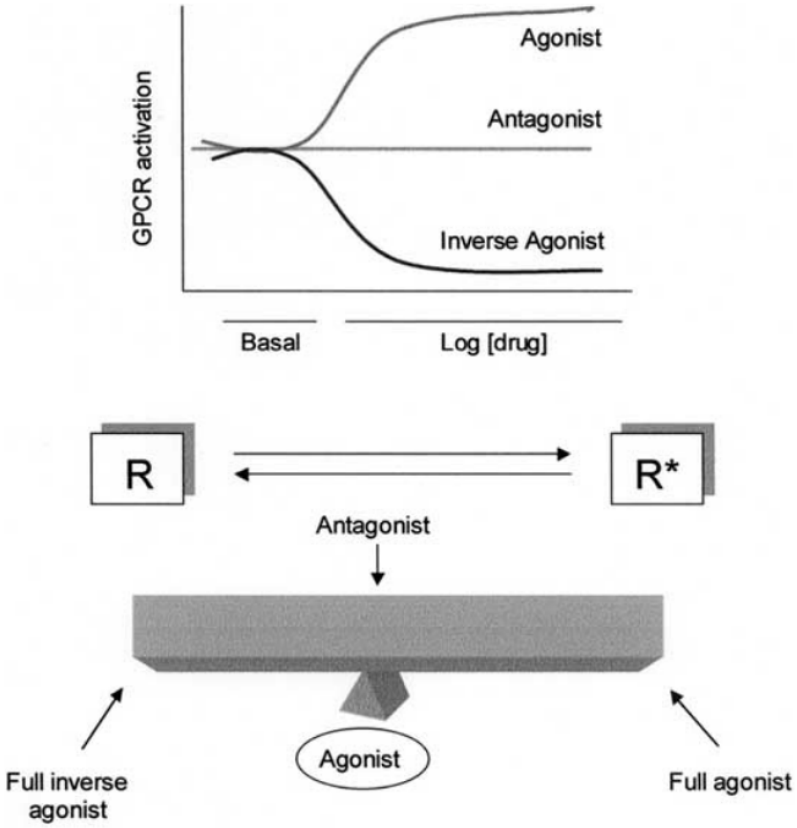
Figure 1.9. Models of receptor occupancy/activation



For the purpose of understanding the activity of the cannabinoids studied in this dissertation, a simplified approach to the cubic ternary model may be taken which omits the considerations of interactions with G-proteins. As illustrated in **Figure 1.10**, it provides

a means to understanding the so-called inverse agonism of some of the compounds discussed. This model may be understood on the basis that, in the absence of ligand, the receptors exist in a dynamic equilibrium of active and inactive states (R vs. R*); this consequently results in some basal level of transduction of second messengers even when a ligand is not present. The efficacy label that we apply to a ligand is a result of the compounds' ability to stabilize either of these states, thereby biasing the equilibrium towards either the active state or the inactive state. Partial to full agonists are therefore defined as compounds that stabilize the active state of the receptor to some degree, and inverse agonists are compounds that stabilize the inactive state. If a ligand stabilizes both states approximately equally, thereby maintaining the basal state of the receptor while simultaneously blocking the active site, it is said to be an antagonist. This model provides a rational theoretical basis to explain why drugs that are considered inverse agonists at a receptor tend to have essentially the opposite physiological effects as agonists at the same receptor (i.e. cannabinoid inverse agonists reduce appetite while agonists increase appetite). [26]

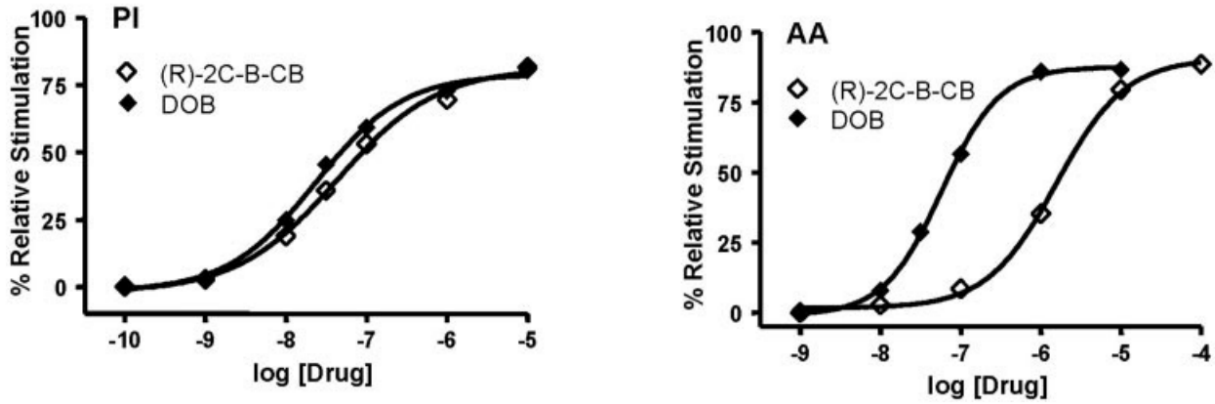
Figure 1.10. Constitutive activity in relation to GPCR dynamics[27]



To further complicate our understanding of GPCR efficacy, recent empirical data has demonstrated that certain GPCRs and ligands exhibit a phenomenon known as functional selectivity, where a single receptor exhibits ligand-dependent differential activation of multiple signal transduction pathways. A key example that is especially relevant to **Chapter 8** of this dissertation is the activity of the functionally selective aminobenzocyclobutene, (R)-2C-B-CB (**8.4a**) at the 5HT2A receptor.[28,29] **Figure 1.11** illustrates functional selectivity via dose-response curves for two related compounds' ability to activate the GPCR 5HT2A with respect to two separate second messengers. The panel on the left displays the dose-response relationship for accumulation of inositol phosphates (PI) and on the right arachadonic acid (AA) release. Looking at the panel on

the left, both compounds have approximately the same efficacy with respect to accumulation of inositol phosphates, whereas (R)-2C-B-CB is nearly 36-fold less efficacious at stimulating AA release while DOB exhibits about the same efficacy relative to PI (right panel). Comparing the two compounds, it could be said that DOB is not a functionally selective ligand, whereas (R)-2B-B-CB is functionally selective with respect to these two second messengers.

Figure 1.11. Comparison of functional selectivity at 5HT2A receptors[28]



With the above example of functional selectivity in mind, once again, the collaboration between pharmacologists and chemists has been paramount in the development of this concept of functional selectivity, which currently exists as the state of the art in pharmacology.

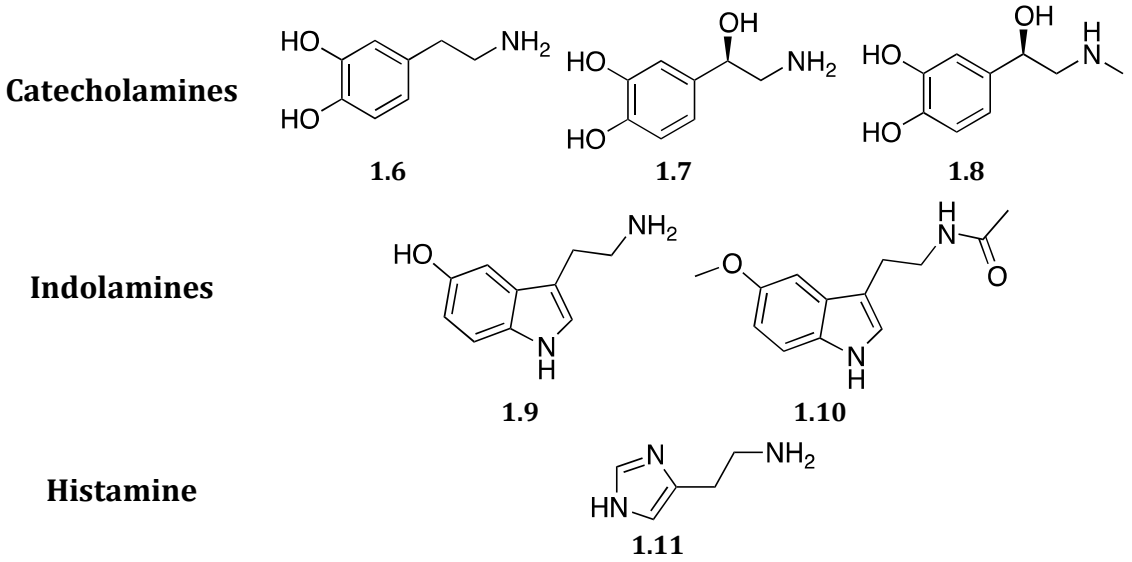
1.3 Biogenic amine neurotransmitters and receptors within the CNS

The biogenic amines function as neurotransmitters in the brain. That is, they are endogenous chemicals that transmit signals from (presynaptic) neurons across synapses

and bind to receptors (usually a GPCR) on target (postsynaptic) neurons.[22] The biogenic amine neurotransmitters mediate a broad scope of mechanisms within the central nervous system, and understanding their role has been an attractive area of research for scientists interested in understanding the brain for many years. Referring back to **Figure 1.2**, most of the early serendipitous discoveries of CNS-active drugs were later found to manipulate one or more of the biogenic amine pathways. Though a complete discussion of the neuroscience of the biogenic amines is well beyond the scope of this introduction, a brief overview will follow with the goal of demonstrating why the manipulation of these neurotransmitters via exogenous chemical influences has the ability to provide valuable insight about the mechanics of the brain and useful pharmacotherapies for treating disorders of the mind.

The biogenic amines relevant to CNS functioning are comprised of three classes of neurotransmitters: the catecholamines, which include dopamine (**1.6**), norepinephrine (**1.7**), and epinephrine (**1.8**); the indolamines, which include serotonin (**1.9**) and melatonin (**1.10**); and finally, histamines (**1.11**). For the purpose of this dissertation and specifically the research described in **Chapter 8**, only CNS mechanisms associated with the neurotransmitters dopamine and serotonin will be discussed in detail.

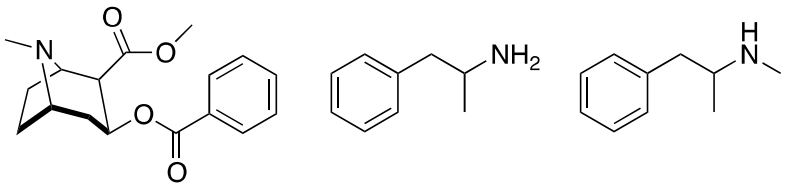
Figure 1.12. The biogenic amine neurotransmitters



In the brain, the cell bodies that primarily communicate via dopamine signalling are found in the midbrain and are comprised of the mesostriatal, mesolimbocortical, periventricular, and tuberohypophyseal systems. Manipulation of these dopaminergic pathways has been found to correlate with behaviors including motor control, cognition, and especially rewarding behaviors associated with sex, thirst, and appetite. It is currently hypothesized that dopaminergic mediation is central to all addictive drugs and behaviors.[30,31] The addictive effects of psychoactive stimulants such as cocaine (**1.12**), amphetamine (**1.13**), and methamphetamine (**1.14**) illustrate prototypical examples of dopamine's role in rewards associated with addiction as all of the drugs have been found to function by effectively increasing the levels of dopamine at the synapse.[32] Consequently, pharmacotherapies with the goal of mitigating the behaviors associated with the disease of addiction will most certainly target dopaminergic pathways in some way.[32] Abnormally low dopamine levels resulting in aberrant dopamine function in nigrostriatal pathways has been highly implicated in the motor deficits associated with Parkinson's disease.[33]

Compounds that block dopamine receptors (antagonists), such as **1.3**, are effective as antipsychotic drugs.[34]

Figure 1.13. Stimulants: cocaine, amphetamine, methamphetamine

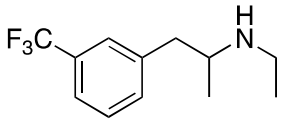


1.12 **1.13** **1.14**

Serotonergic cells are primarily centralized in two clusters within the brain: the caudal system within the medulla and the rostral system in the midbrain. These cells then project throughout the rest of the CNS, suggesting that serotonergic signaling is far-reaching and mediates communication across many regions of the brain.[35] Serotonin is the primary neurotransmitter associated with behaviors relating to appetite, sleep, and aggression ("mood" in humans).[36] It has been found that serotonin reuptake blockers such as fenfluramine, (**1.15**), which increase serotonin at the synapse, are effective in significantly reducing appetite.[37] Conversely, decreased levels of serotonin at the synapse are generally associated with increased aggressiveness.[38] Measurements of levels of serotonin metabolites in the cerebrospinal fluid and blood of animals and humans can be an accurate predictor of a history of aggressive behavior.[39,40] Considerable attention has also been focused on the production of drugs that modulate serotonergic signaling for the purpose of treating depression. Once again, referring back to **Figure 1.3**, many of the early drugs that were found to be useful in easing the symptoms associated with depression

(TCAs, SSRIs, MAOIs) were later found to modulate serotonergic circuits in one or more ways.

Figure 1.14. Fenfluramine, an appetite-reducing serotonin-increasing compound



1.15

Unfortunately, the analysis above paints an incomplete and overly simplistic picture of the nature of biogenic amine neurotransmitters. The story is complicated significantly by the fact that the GPCRs (and other proteins) to which these neurotransmitters bind are quite diverse, existing as a multitude of related yet functionally distinct subtypes that are fundamentally interwoven physiologically.[36,41,42] Furthermore, the emerging evidence that these neurotransmitters can also be modulated by "third party" cannabinoids (discussed below) further complicates the puzzle.[43–46] Therefore, part of our motivation in producing the compounds described in the subsequent chapters was to provide additional tools to hopefully aid in the continued elucidation of these central mechanisms of the brain.

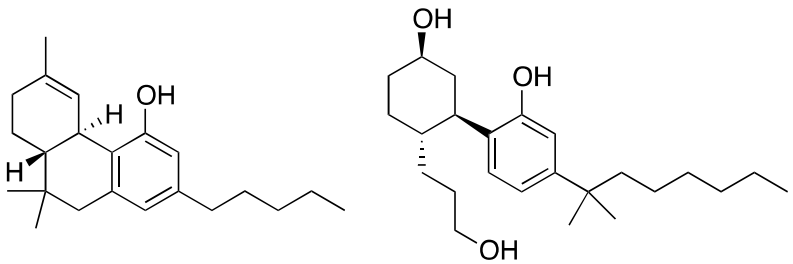
1.4 Cannabinoids and endocannabinoids

Cannabinoids are molecules that selectively bind to a unique class of G-protein coupled receptors. To date, two subclasses of cannabinoid receptor, CB1 and CB2, have been studied in detail. Additional subtypes of the cannabinoid receptor have been

hypothesized to exist, but are not yet well understood or thoroughly supported experimentally.[47–49] Cannabinoids and their receptors function in the cell by a unique mechanism known as retrograde signaling where, in contrast to classical neurotransmitters, cannabinoids are synthesized in the post-synaptic neuron and bind to receptors presynaptically in order to suppress neurotransmitter release.[50]

Though humans have consumed the cannabis plant for thousands of years, our scientific understanding of the receptor system that interacts with the plant's pharmacologically active constituents has only recently begun to be elucidated, and cannabinoids currently represent a new frontier in understanding one of the fundamental mechanisms of the brain. In 1964, Δ^9-tetrahydrocannabinol (THC, **1.16**), the primary active component of cannabis, was isolated and synthesized by Gaoni and Mechoulam.[51] At the time, it was believed that the highly lipophilic molecule interacted nonspecifically with cell membranes. Not until twenty years later in the 1980's did Howlett & Fleming publish data which indicated that the receptor that responds to Δ^9-THC may be a G-protein-coupled receptor.[52] However, further elucidation of the receptor was impeded due to the lipophilic nature of THC. This problem was circumvented by Pfizer's development of a more hydrophilic THC synthetic analog, CP-55,940 (**1.17**).

Figure 1.15. Plant-derived Δ^9-tetrahydrocannabinol and the hydrophilic synthetic analog, CP-55,940

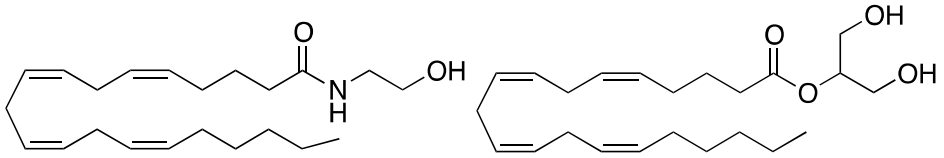


1.16 **1.17**

Using radiolabeled CP-55,940 binding site distribution data combined with the pattern of mRNA expression of a specific GPCR, the first cannabinoid receptor was definitively identified in the rat brain in 1990.[53] A second receptor was discovered in 1993 by The Munro group with 44% sequence similarity that exhibited binding affinity for Δ^9-THC and CP-55,940; this receptor was found to be primarily expressed in the immune system, but not the brain. In order to distinguish between the two receptors, Munro and colleagues coined the brain and immune receptors CB1 and CB2, respectively, in 1993.[54]

Despite the mounting data indicating the existence of a receptor system activated by plant-derived Δ^9-THC, it would be reasonable to assume that the endocannabinoid system did not evolve for the purpose of cannabis consumption. Indeed, not long after the two cannabinoid receptors were identified, Mechoulam (who determined the structure of THC in 1964) and several others independently isolated the putative endogenous cannabinoid receptor ligands, anandamide (**1.18**, Sanskrit for "bliss") and 2-arachidonyl glycerol (**1.19**, 2-AG).[55–57] Although anandamide and 2-AG are the most well studied endocannabinoids, many other endogenous molecules have been implicated in cannabinoid signaling. These molecules generally consist of amides, esters, and ethers of long chain polyunsaturated fatty acids.[58]

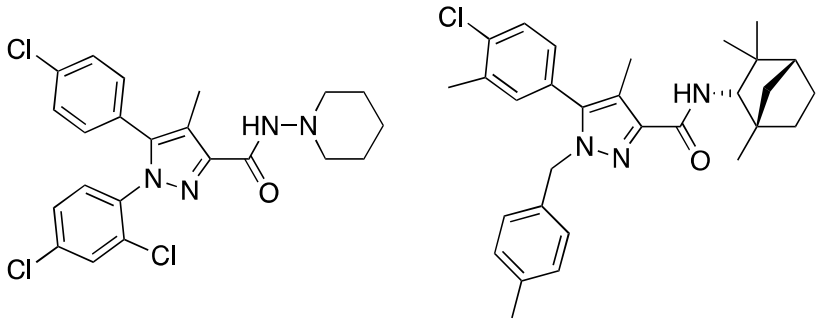
Figure 1.16. Anandamaide and 2-arachidonyl glycerol, the endocannabinoids



1.18 **1.19**

The development of selective agonists and antagonists for CB1 and CB2 allowed researchers to definitively distinguish between the two receptors. Synthesized by Sanofi Recherche, SR141716A (**1.20**) was the first CB1 selective inverse agonist. This compound demonstrated nanomolar affinity for CB1 but only micromolar affinity for CB2.[59] Four years later, SR144528 (**1.21**) was developed. Compound **1.21** has a 700-fold lower affinity for CB1 than CB2.[60] Thus, both receptors could be studied in detail independently.

Figure 1.17. SR141716A and SR144528, selective CB1 and CB2 receptor ligands

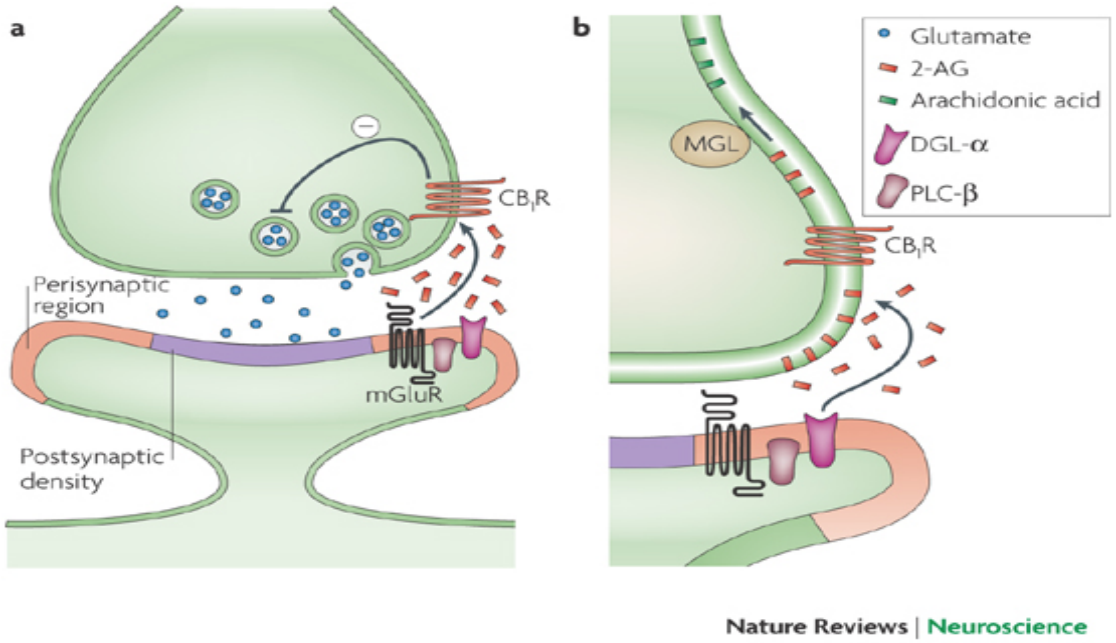


1.20 **1.21**

The role of cannabinoids in the brain is complex and many psychological, behavioral, and physical processes are implicated. Cannabinoids are believed to function as "homeostasis regulators" in the body.[61] Endocannabinoids are synthesized on-demand in post-synaptic neurons and bind to cannabinoid receptors on pre-synaptic neurons, thereby tuning down, or attenuating, the rate of anterograde neurotransmission. As illustrated in **Figure 1.18**, the endocannabinoid 2-AG is shown to regulate glutamate neurotransmission by forming a negative feedback loop. Glutamate is released presynaptically and binds to receptors on the postsynaptic neuron, which in turn activates enzymes that increase endocannabinoid synthesis from membrane lipids. The endocannabinoids are then released into the synapse where they bind to cannabinoid

receptors on the presynaptic neuron. The cannabinoid receptor in turn transduces a signal to attenuate glutamate vesicle packaging, thereby regulating glutamate release.

Figure 1.18. Schematic of cannabinoid retrograde signaling at the synapse[62]



CB1 receptors are expressed throughout much of the CNS and therefore mediate a range of physiological functions.[63] As recent evidence has demonstrated, the activation of CB1 receptors inhibits the synaptic release of many neurotransmitters including γ-aminobutyric acid, glutamate, acetylcholine, and the monoamines.[45] The indirect regulation of monoamine neurotransmitters such as serotonin and dopamine by way of cannabinoid function holds particular promise for the development of new theories about the brain, and in turn, novel pharmacotherapies to treat disorders of the mind, given the significant behavioral implications of these neurotransmitters as described in **Section 1.3**.

The therapeutic benefits of cannabinoid-mediated therapies have recently begun to be realized. Cannabinoids generally exhibit low toxicity and demonstrate great potential for medicinal utility.[64] Current cannabinoid based medications generally target the peripheral (non-psychoactive) CB2 receptor. These medications include treatments for nausea and vomiting associated with chemotherapy, muscle spasticity associated with multiple sclerosis, alleviation of chronic pain, and glaucoma.[65] Given the recent findings in the cannabinoids' ability to regulate dopaminergic function, CB1 receptor ligands have become attractive targets for addiction pharmacotherapy.[66] With this in mind, the research in the subsequent chapters was initiated in order to further explore this new frontier in the study of the mind and potentially develop compounds that may be medicinally useful in treating disorders of the mind by way of modulation of cannabinoid receptors.

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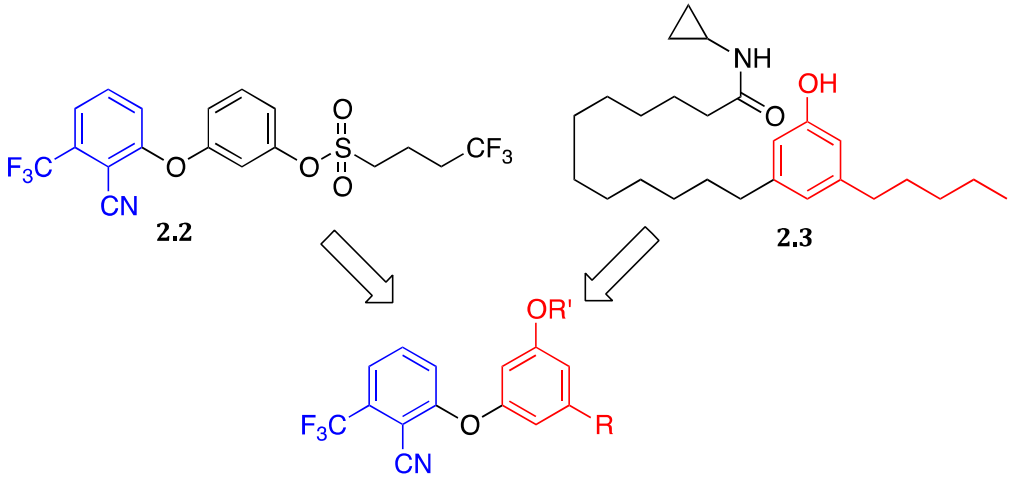
2

Diaryl Ether Hybrid Analogs as Cannabinoid Receptor Ligands – Rationale, Synthesis and Preliminary Data

2.1 Abstract

Preliminary data regarding the synthesis and utility of diaryl ether derivatives as cannabinoid receptor ligands is reported. Initially, we sought to prepare hybrid analogs of BAY 59-3074 and CB25 in order to exploit the favorable properties of each molecule. Both compounds have demonstrated partial agonist activity at the CB1 receptor. Molecular features of each compound were combined to synthesize hybrid derivatives in order to evaluate their pharmacological potential. Several moderate affinity compounds emerged and indicated that hybrid diaryl ethers may be viable as potential cannabinoid receptor ligands.

Scheme 2.1. Abstract scheme: BAY 59-3074 / CB 25 hybrid analogs

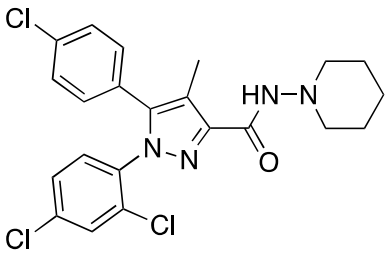


2.2 Introduction

The initial goal of this project was to develop novel high affinity low efficacy (neutral antagonist to partial agonist) CB1 receptor ligands. These compounds will be useful for exploring the (relatively unexplored) cannabinoid pharmacophore and specifically represent potentially attractive targets for addiction pharmacotherapy.[1,2] While cannabinoid inverse agonist pharmacotherapies have been explored somewhat rigorously, neutral antagonist/partial agonist studies are more sparse, partially due to limited availability of useful compounds. Cannabinoid inverse agonists have been implicated in being potentially useful for the treatment of a variety of disorders including obesity and addiction, but they tend to have undesirable side effects that include nausea, increased nociceptive activity, increased anxiety and depression.[3–6] Many of these side effects were discovered in the clinical trials for the cannabinoid inverse agonist Rimonabant **(2.1)**.[7] It has been hypothesized that that low efficacy agonists/neutral antagonists may diminish some of the negative properties seen with inverse agonists, especially nausea, while retaining some of the desirable qualities of cannabinoid targeted pharmacotherapies.[8] To

date, there are very few high affinity CB1 selective ligands that exhibit low efficacy profiles, and therefore, less is known about the CB1 partial agonist pharmacophore. The research described in the present study was initiated with the goal of making novel high affinity, low efficacy cannabinoids available to the research community for further study.

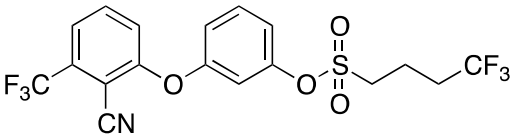
Figure 2.1. Cannabinoid inverse agonist Rimonabant (SR141716)



2.1

The compounds BAY 59-3074 (**2.2**) and CB25 (**2.3**) exhibit low efficacy agonist profiles both *in vitro* and *in vivo* as compared to THC. BAY 59-3074 was discovered by Bayer Pharmaceutical by screening compound libraries, and so a full SAR of this compound was not well established; this compound has only modest affinity at the CB1 receptor with a K_i value of 48.3 nM. The compound has demonstrated good bioavailability and blood-brain barrier (BBB) penetrability.[9]

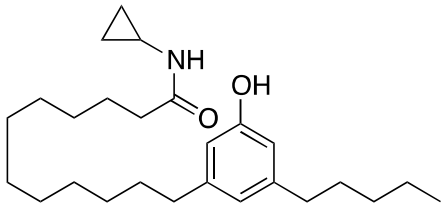
Figure 2.2. BAY 59-3074, a CB1 receptor partial agonist



2.2

The olivetol derivative CB25 was introduced by the DiMarzo *et al.* and exhibits high affinity (K_i = 5nM) at CB receptors.[10,11] CB25 is, however, very lipophilic (ClogP = 6.2) and is unlikely to be a viable drug candidate.

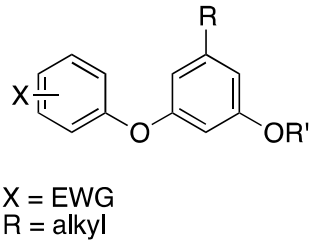
Figure 2.3. CB25, a CB1 receptor partial agonist



2.3

With both of these compounds serving as leads, it was envisaged that perhaps a "hybrid derivative" could be developed in order to exploit the desirable properties of each molecule. To improve the hydrophilicity and take advantage of the high potency of CB25 the amide side chain of CB25 was replaced with an aryl moiety similar to BAY 59-3074. We therefore sought to develop a synthetic methodology in order to produce compounds belonging to the scaffold shown in **Figure 2.4** with general features including an electron-deficient aryl ring coupled to an aryl system bearing hydrophobic alkyl chains and oxygen linkages.

Figure 2.4. Preliminary ligand design strategy

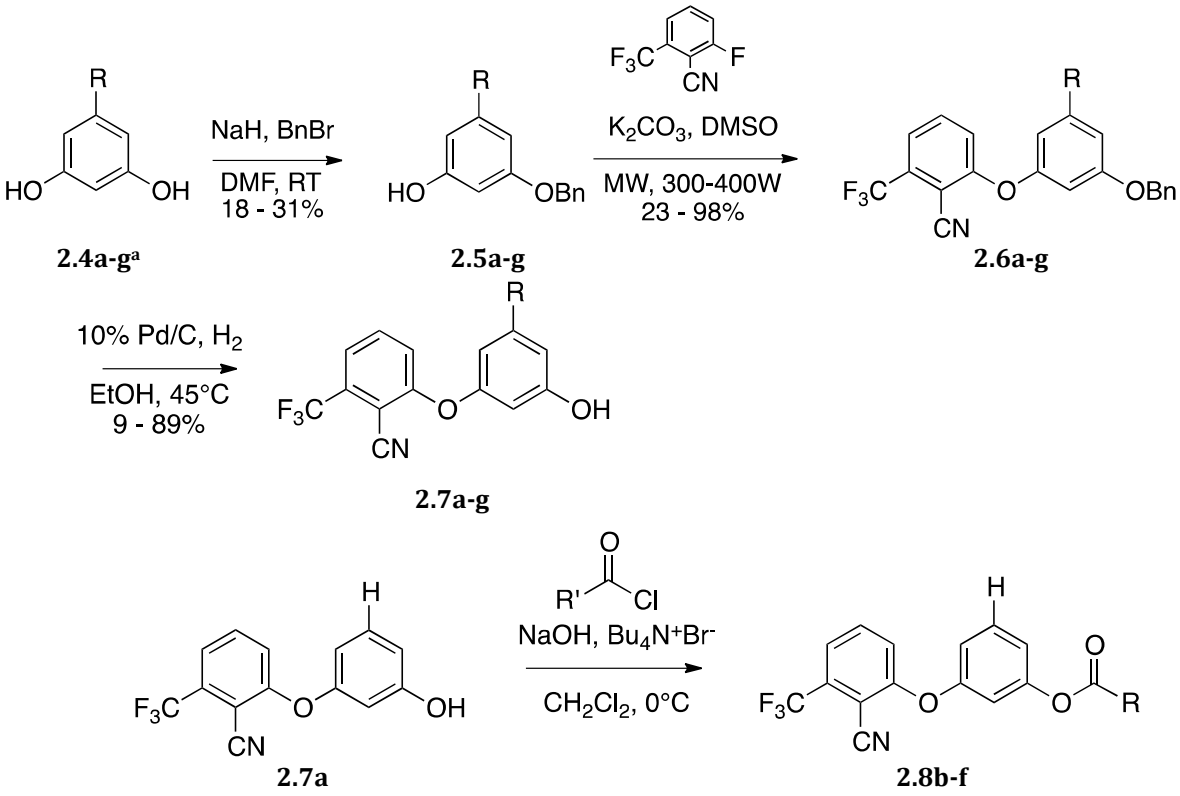


2.3 Results and Discussion

The synthesis of target compounds was achieved in good to moderate yield and the details of these procedures have been described by our group elsewhere.[12] Our general synthetic strategy is outlined in **Scheme 2.2** and began with mono-protection of select alkyl-resorcinols **2.4a-g** via benzylation using benzyl bromide and sodium hydride. The alkyl-resorcinol precursors **2.4a-g** were either commercially available or prepared according to previously established methods.[12] Given the symmetry of these resorcinol compounds, a mixture of mono-benzylation and di-benzylation product was produced despite the use of only one stoichiometric equivalent of base, and so unfortunately only moderate yields of this early precursor could be obtained. Mono-protected alkyl-resorcinols **2.5a-g** were coupled to the electron-deficient aryl fluoride via a microwave-assisted nucleophilic aromatic substitution to produce the corresponding diaryl ethers **2.6a-g** in good yield following the protocol established by the Wang group.[13] Debenzylation via hydrogenolysis removed the benzyl protecting group and afforded the key diaryl ether scaffolds **2.7a-g**.

The phenolic site of diaryl ether **2.7a** was subject to further modification in order to expand our preliminary SAR. We hypothesized that introducing an ester at this position could possibly produce high affinity compounds that more closely mimic the structure of BAY 59-3074. Subjecting **2.7a** to phase transfer catalysis conditions and an appropriate acid halide afforded compounds **2.8b-f** as diaryl ether esters.

Scheme 2.2. Synthetic strategy for the synthesis of target compounds

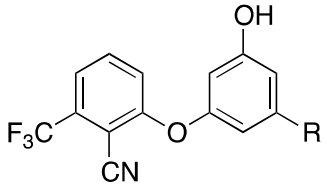


[a]for suffixs **a-g**, R = H, methyl, ethyl, propyl, butyl, pentyl, hexyl, respectively.

Tables 2.1 and **2.2** represent a summary of the available bioassay data for the first generation of hybrid derivative diaryl ethers synthesized. Compounds **2.7d**, **2.7e**, **2.7f**, **2.6a**, **2.8e** demonstrated approximate micromolar affinity for the CB1 receptor, but no high

affinity nanomolar compounds emerged from the present studies. The results were, however, encouraging enough to carry on with the SAR experiments and synthesize more compounds. A full analysis and discussion of the bioassay data from **Tables 2.1** and **2.2** is presented in **Chapter 6** within the context of the entire diaryl ether SAR studies.

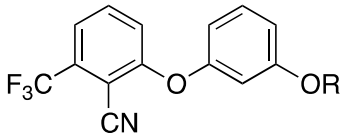
Table 2.1. *In vitro* binding data at CB1 and [^{3}H]SR141716A inhibition for alkyl resorcinol series



Cpd	Code	R	CB1[b] (1 μM)	CB1[b] (10 μM)	[3H]SR141716A (CB1) K_i (nM)[a]
2.7a	ARN142	H	3	37	
2.7b	ARN156	CH_3	16	30	21,055±3217
2.7c	ARN167	CH_2CH_3	21	37	
2.7d	ARN180	$CH_2CH_2CH_3$	24	60	
2.7e	ARN191	$CH_2(CH_2)_2CH_3$	35	94	
2.7f	ARN158	$CH_2(CH_2)_3CH_3$	39	41	293±107
2.7g	ARN190	$CH_2(CH_2)_4CH_3$	nd	nd	

[a] All the values are mean ± SEM of three experiments. [b] Binding affinities at CB1 receptor measured as % inhibition.

Table 2.2. *In vitro* binding data at CB1 and [3H]SR141716A inhibition for alkyl ester series



Cpd	Code	R	CB1[b] (1 μM)	CB1[b] (10 μM)	[3H]SR141716A (CB1) K_i (nM)[a]
2.6a	AS109	Bn	49	56	
2.8b	ARN199	$COCH_3$	18	45	
2.8c	ARN200	$COCH_2CH_3$	25	34	
2.8d	ARN201	$CO(CH_2)_2CH_3$	25	25	
2.8e	ARN202	$CO(CH_2)_3CH_3$	25	49	
2.8f	ARN203	$CO(CH_2)_4CH_3$	7	19	

[a] All the values are mean ± SEM of three experiments. [b] Binding affinities at CB1 receptor measured as % inhibition.

2.4 Conclusion

A series of BAY 59-3074 / CB 25 hybrid derivatives were synthesized to obtain a new class of diaryl ether CB1 receptor ligands. Alkyl ligands **2.6a-2.6g** and ester ligands **2.7b-2.7f** were synthesized in good to moderate yield and subsequently evaluated for binding affinity at the CB1 receptor. Several compounds with micromolar affinity for the receptor were discovered as a result. Future SAR studies will focus on further manipulation of the diaryl ether scaffold for the development of novel high affinity CB1 receptor ligands.

2.5 Acknowledgement

This research was funded by the National Institute on Drug Abuse (DA023916) and the University of New Orleans.

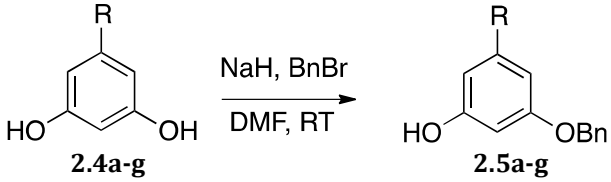
The synthesis and characterization of the compounds described in this chapter were accomplished in collaboration with Dr. April Noble-Brooks. See reference 12 for further details.

2.6 Experimental Section

General Methods

All chemicals were purchased from Aldrich Chemical Company unless otherwise noted. Anhydrous toluene, tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), and methanol (MeOH) were purchased from Mallinckrodt Baker, Inc and were used under nitrogen without any further purification. Thin layer chromatography (TLC) 20 x 20 cm glass plates precoated with 250 μm silica gel were purchased from Sorbent Technologies and used to monitor reactions via visualization with short-wave UV light, iodine, potassium permanganate, phosphomolybdic acid (PMA), 2,4-dinitrophenyl hydrazine or Dragondorff's reagent. Chromatography is in reference to column chromatography on silica gel (Silica Gel 60, 230-400 mesh). High-pressure hydrogenations were carried out on a Parr apparatus. Proton and carbon NMR were recorded on a Varian-Gemini 300 and 400 MHz nuclear magnetic resonance spectrometer, respectively at ambient temperature in deuterated chloroform from Cambridge Isotope Laboratories, Inc. ^1H NMR chemical shifts

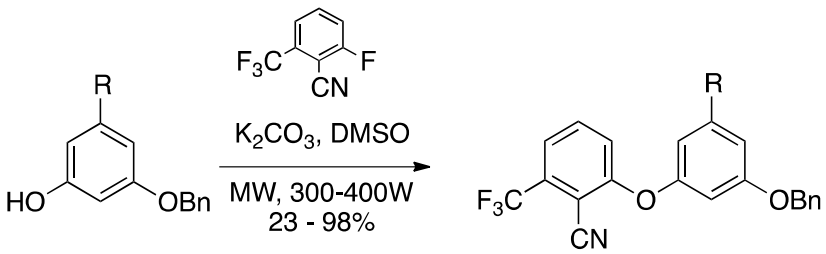
are reported in δ values (ppm) with tetramethylsilane (TMS), employed as the internal standard. ^{13}C NMR chemical shifts are reported in δ values (ppm) with chloroform-D ($CDCl_3$, 77.0 ppm), employed as the internal standard. Elemental analyses were obtained from Atlantic Microlabs, Inc.



2.4a-g **2.5a-g**

General Method A: Preparation of monobenzylated 1,3-diols

The appropriate alkyl resorcinol **2.4a-g** (1 equiv.) in DMF was added dropwise to NaH (1 equiv.) in DMF. The reaction mixture was allowed to stir for 30 minutes at room temperature. BnBr (1 equiv.) in DMF was added dropwise to the reaction mixture and allowed to stir for 2 h at room temperature. Distilled H_2O was added and the mixture was extracted with EtOAc. The organic layers were combined, washed with 0.5 N HCl, washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure.

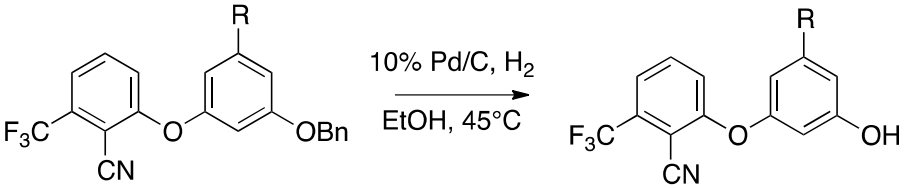


2.5a-g **2.6a-g**

General Method B: Preparation of Diaryl Ethers

2-Fluoro-6-(trifluoromethyl)benzonitrile (1 equiv.), alkyl phenol **2.5a-g** (1 - 1.2 equiv.), and potassium carbonate (2 equiv.) were added to DMSO. Using a microwave power of 300-400

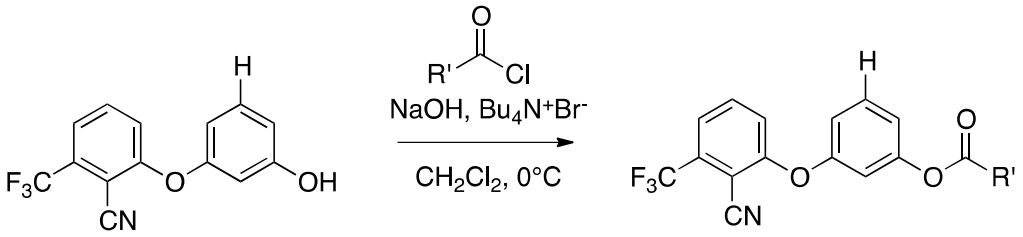
W the temperature was ramped from room temperature to the boiling point of DMSO. Upon completion of the reaction, it was cooled to room temperature, put into ice water, and extracted with Et_2O. The organic layers were combined, washed with 0.5 N HCl, washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure.



2.6a-g **2.7a-g**

General Method C: Preparation of Debenzylated Diaryl Ethers

A solution of O-benzyl diaryl ether **2.6a-g** (1 equiv.) and 10% Pd/C (50 wt. %) in EtOH (23 mL) was hydrogenated using a hydrogen balloon at 45 °C. The reaction mixture was filtered through celite and evaporated to dryness under reduced pressure.

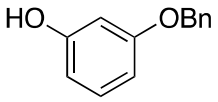


2.7a **2.8b-f**

General Method D: Preparation of Esters

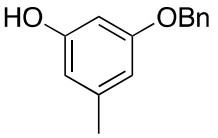
Under argon, the phenol **2.7a** (0.53 mmol) was dissolved in DCM and tetrabutylammonium iodide (0.27 mmol) and 20M NaOH were added and stirred. At 0 °C, the acid halide (0.63 mmol) dissolved in DCM was added. The reaction mixture was allowed to stir for 1 h after the color change at 0 °C and for another 1h at room temperature. The reaction mixture was

diluted with water and extracted with DCM. The organic layers were collected, washed with brine, and dried over $MgSO_4$.



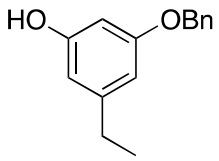
3-Benzyloxyphenol (2.5a)[14,15]

General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:5, 1:4) to afford **2.5a** (0.86 g, 24%) as an amber oil. TLC: R_f 0.46 (EtOAc/hexane, 1:4): ^{1}H NMR (400 MHz; $(CD_3)_2CO$) δ 4.75 (broad s, 1, OH), 5.04 (s, 2H), 6.43 (tt, J = 1.6 Hz, 2.0 Hz, 1H), 6.49 (t, J = 2.4 Hz, 1H), 6.56 (qq, J = 0.8 Hz, 0.8 Hz, 1H), 7.14 (t, J = 8.4 Hz, 1H), 7.31-7.44 (m, 5H). ^{13}C NMR (100 MHz; $(CD_3)_2CO$) δ 69.7, 102.6, 106.3, 108.4, 127.8, 128.0(2), 128.7(2), 130.3, 137.8, 158.9, 160.6.



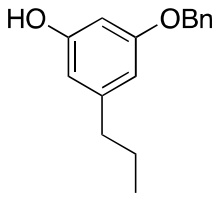
3-Benzyloxy-5-methyl-phenol (2.5b)[14,15]

General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:5, 1:4) to afford **2.5b** (1.0 g, 29%) as a pale yellow oil. TLC: R_f 0.44 (EtOAc/hexane, 1:4): ^{1}H NMR (400 MHz; $(CD_3)_2SO$) δ 2.18 (s, 3H), 5.00 (s, 2H), 6.24 (d, J = 4.4 Hz, 2H), 6.29 (s, 1H), 7.31 (t, J = 4.0 Hz, 3H), 7.35-7.43 (m, 2H), 9.30 (broad s, 1, OH). ^{13}C NMR (100 MHz; $(CD_3)_2SO$) δ 21.3, 69.0, 99.4, 106.4, 108.9, 127.6(2), 127.7, 128.4(2), 137.4, 139.5, 158.5, 159.6.



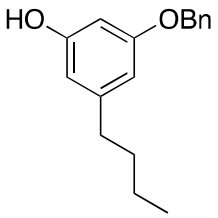
3-Benzyloxy-5-ethylphenol (2.5c) [14,15]

General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.5c** (0.379 g, 18%) as a yellow oil. TLC: R_f 0.47 (EtOAc/hexane, 1:4): ^1H NMR (400 MHz; $CDCl_3$) δ 1.21 (t, J = 7.6 Hz, 3H), 2.57 (q, J = 7.6 Hz, 2H), 4.61 (broad s, 1, OH), 5.02 (s, 2H), 6.31 (q, J = 2.4 Hz, 1H), 6.44 (s, 2H), 7.33-7.44 (m, 5H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 15.5, 29.1, 70.2, 99.7, 107.4, 107.8, 127.8(2), 128.2, 128.8(2), 137.2, 147.4, 156.7, 160.3.



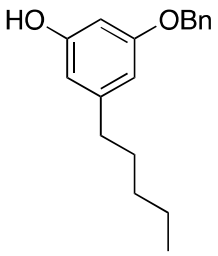
3-Benzyloxy-5-propylphenol (2.5d) [14,15]

General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.5d** (0.256 g, 19%) as a yellow oil. TLC: R_f 0.51 (EtOAc/hexane, 1:4): ^1H NMR (400 MHz; $CDCl_3$) δ 0.93 (t, J = 7.2 Hz, 3H), 1.50-1.66 (m, 2H), 2.53 (t, J = 7.2 Hz, 2H), 5.02 (s, 2H), 6.23 (d, J = 2.0 Hz, 1H), 6.23-6.47 (m, 2H), 7.09-7.44 (m, 5H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 13.9, 24.4, 38.2, 70.5, 104.6, 112.9, 113.0, 127.8(2), 128.4, 128.9(2), 136.6, 146.7, 160.4, 161.9.



3-Benzyloxy-5-butylphenol (2.5e) [14,15]

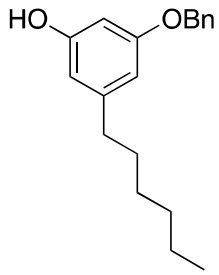
General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane, 1:9, 1:7, 1:5) to afford **2.5e** (0.409 g, 26%) as a dark brown oil. TLC: R_f 0.67 (EtOAc/hexane, 3:7): ^1H NMR (400 MHz; $CDCl_3$) δ 1.03-1.08 (m, 3H), 1.42-1.51 (m, 2H), 1.64-1.72 (m, 2H), 2.62 (dd, J = 7.6 Hz, 2.0, 2H), 5.07 (s, 2H), 6.38 (d, J = 4.4 Hz, 1H), 6.44 (s, 1H), 6.48 (d, J = 2.4 Hz, 1H), 6.49 (s, 1H), 7.26-7.53 (m, 5H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.3, 22.7, 33.6, 36.1, 70.5, 100.1, 108.4, 108.9, 128.0(2), 128.4, 129.0(2), 137.3, 146.2, 156.7, 160.3.



3-Benzyloxy-5-pentyl-phenol (2.5f) [14,15]

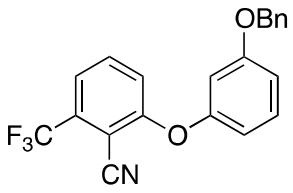
General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:5, 1:4) to afford **2.5f** (0.457 g, 31%) as a brown oil. TLC: R_f 0.53 (EtOAc/hexane, 1:4): ^1H NMR (400 MHz; $(CD_3)_2CO$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.26-1.34 (m, 4H), 1.55-1.63 (m, 2H), 2.49-2.56 (m, 2H), 5.02 (s, 2H), 7.30-7.44 (m, 5H), 8.21 (broad s, 1,

OH). ^{13}C NMR (100 MHz; $(CD_3)_2CO$) δ 13.7, 22.6, 31.1, 31.6, 36.0, 69.6, 99.8, 106.5, 108.4, 127.7(2), 127.9, 128.6(2), 138.0, 145.3, 158.6, 160.4.



3-Benzyloxy-5-hexylphenol (2.5g) [14,15]

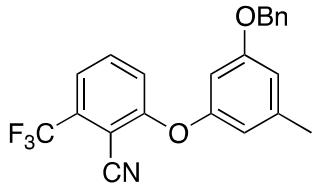
General Method A. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.5g** (0.374 g, 21%) as a light brown oil. TLC: R_f 0.72 (EtOAc/hexane, 3:7): ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.95 (dt, J = 2.4 Hz, 4.0 Hz, 3H), 1.31-1.40 (m, 6H), 1.62 (q, J = 7.6 Hz, 2H), 2.56 (t, J = 8.0 Hz, 2H), 5.03 (s, 2H), 5.75 (broad s, 1, OH), 6.36 (d, J = 8.8 Hz, 1H), 6.38 (t, J = 2.4 Hz, 1H), 6.50 (d, J = 1.2 Hz,1H), 7.35-7.48 (m, 5H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.3, 22.8, 29.2, 31.2, 31.9, 36.2, 70.2, 99.9, 108.0, 108.6, 127.8(2), 128.2, 128.8(2), 137.1, 146.0, 156.6, 160.1.



2-(3-Benzyloxy-phenoxy)-6-trifluoromethyl-benzonitrile (2.6a)[16]

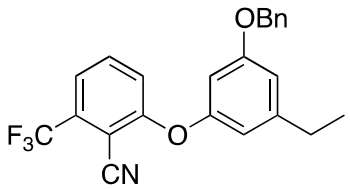
General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:10, 1:9, 1:4) to afford **2.6a** (0.325 g, 23%) as a white solid. TLC: R_f 0.60 (EtOAc/hexane, 1:4): ^{1}H NMR (400 MHz; $(CD_3)_2SO$) δ 5.16 (s, 2H), 6.82 (t,

J = 8.4 Hz, 1H), 6.94-7.00 (m, 2H), 7.24 (d, J = 11.6 Hz, 1H), 7.33-7.45 (m, 6H), 7.65 (d, J = 10.0 Hz, 1H), 7.82 (d, J = 10.8 Hz, 1H). ^{13}C NMR (100 MHz; $(CD_3)_2SO$) δ 70.1, 107.2(3), 112.5, 112.8, 120.8(2), 121.1, 127.8(3), 128.1(2), 128.7, 131.2, 135.2, 137.2, 155.9, 160.9, 161.0. MS (ESI) m/z 392.2 (M + Na), 387.3 (M + H_2O), 370.2 (M + 1). Anal. Calcd. For $C_{21}H_{14}F_3NO_2$: C, 68.29; H, 3.82; N, 3.79. Found: C, 68.21; H, 3.77; N, 3.81.



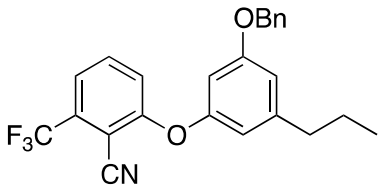
2-(3-Benzyloxy-5-methyl-phenoxy)-6-trifluoromethyl-benzonitrile (2.6b)[16]

General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:5, 1:4) to **2.6b** afford (1.35 g, 91%) as a pale yellow oil. TLC: R_f 0.56 (EtOAc/hexane, 1:5): ^{1}H NMR (400 MHz; $(CD_3)_2CO$) δ 2.34 (s, 3H), 5.14 (s, 2H), 6.66 (s, 1H), 6.73 (s, 1H), 6.84 (s, 1H), 7.25 (d, J = 8.8 Hz, 1H), 7.34 (d, J = 7.2 Hz, 1H), 7.39 (t, J = 7.2 Hz, 2H), 7.46 (d, J = 7.2 Hz, 2H), 7.65 (d, J = 7.6 Hz, 1H), 7.82 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100MHz; $(CD_3)_2CO$) δ 20.9, 70.1, 104.3(2), 113.2, 113.5, 120.7(2), 120.8, 121.1, 127.8(2), 128.1, 128.7(2), 135.2(2), 137.3, 141.9, 155.7, 160.6, 161.7.



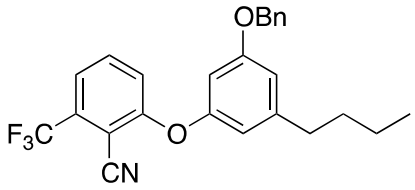
2-(3-(Benzyloxy)-5-ethylphenoxy)-6-(trifluoromethyl)benzonitrile (2.6c)[16]

General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.6c** (0.404 g, 74%) as a pale yellow oil. TLC: R_f 0.71 (EtOAc/hexane, 1:4): ^1H NMR (400 MHz; $CDCl_3$) δ 1.23 (t, J = 7.6 Hz, 3H), 2.63 (q, J = 7.2 Hz, 2H), 5.04 (s, 2H), 6.53 (t, J = 2.4 Hz, 1H), 6.56 (s, 1H), 6.75 (s, 1H), 7.06 (d, J = 8.8 Hz, 1H), 7.32-7.44 (m, 2H), 7.53 (t, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 15.4, 29.1, 70.5, 104.5(2), 112.4(2), 112.5, 120.1, 120.2(2), 127.8(2), 128.4, 128.9(2), 134.0(3), 148.2, 155.4, 160.5, 161.8.



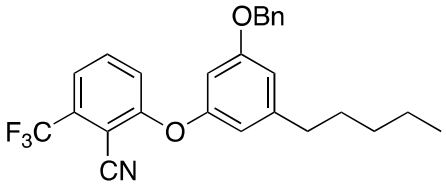
2-(3-(Benzyloxy)-5-propylphenoxy)-6-(trifluoromethyl)benzonitrile (2.6d)[16]

General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.6d** (0.279 g, 64%) as a pale yellow oil. TLC: R_f 0.63 (EtOAc/hexane, 1:4): ^1H NMR (400 MHz; $CDCl_3$) δ 0.93 (t, J = 7.6 Hz, 3H), 1.60-1.66 (m, 2H), 2.56 (t, J = 7.2 Hz, 2H), 5.04 (s, 2H), 6.54 (d, J = 1.6 Hz, 2H), 6.73 (s, 1H), 7.05 (d, J = 8.8 Hz, 1H), 7.32-7.44 (m, 6H), 7.53 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 13.4, 24.4, 38.2, 70.5, 95.7, 104.6(2), 112.9, 113.0, 115.6, 120.0, 120.1(2), 127.8(2), 128.3, 128.9(2), 134.0, 136.6, 146.7, 155.3, 160.4, 161.9.



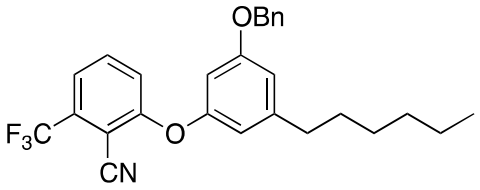
2-(3-(Benzyloxy)-5-butylphenoxy)-6-(trifluoromethyl)benzonitrile (2.6e)[16]

General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane 3:7, CH_2Cl_2/Hexane 3:7) to afford **2.6e** (0.244 g, 36%) as a colorless oil. TLC: R_f 0.80 (CH_2Cl_2/hexane, 3:7): ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.94 (t, J = 7.6 Hz, 3H), 1.28-1.41 (m, 2H), 1.56-1.64 (m, 2H), 2.61 (t, J = 6.8 Hz, 2H), 5.05 (s, 2H), 6.54-6.56 (m, 2H), 6.75 (s, 1H), 7.07 (d, J = 8.8 Hz, 1H), 7.32-7.45 (m, 6H), 7.53 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.1, 22.5, 33.4, 35.9, 70.5, 104.6(2), 112.6, 112.9, 113.0, 120.1, 120.2(2), 127.8(2), 128.4, 128.9(2), 134.1(2), 136.7, 146.9, 155.3, 160.4, 161.9.



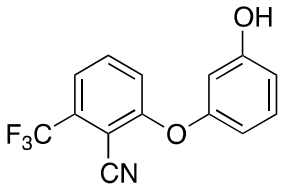
2-(3-Benxyloxy-5-pentyl-phenoxy)-6-trifluoromethyl-benzonitrile (2.6f)[16]

General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.6f** (0.446 g, 72%) as a pale yellow oil. TLC: R_f 0.36 (EtOAc/hexane, 1:9): ^{1}H NMR (400 MHz; $(CD_3)_2CO$) δ 0.88 (dd, J = 3.2 Hz, 6.8 Hz, 3H), 1.29-1.36 (m, 4H), 1.63 (q, J = 5.6 Hz, 2H), 2.62 (t, J = 8.0 Hz, 2H), 5.14 (s, 2H), 6.69 (t, J = 2.0 Hz, 1H), 6.75 (t, J = 2.4 Hz, 1H), 6.86 (t, J = 2.0 Hz, 1H), 7.24 (d, J = 8.8 Hz, 1H), 7.33-7.41(m, 3H), 7.46-7.48 (m, 2H), 7.63 (d, J = 7.6 Hz, 1H), 7.79-7.83 (m, 1H). ^{13}C NMR (100 MHz; $(CD_3)_2CO$) δ 13.7, 30.9, 31.5, 32.5, 35.8, 70.1, 104.5(2), 112.7(2), 113.0, 120.6(2), 120.9, 127.8(2), 128.1, 128.7(2), 135.2(2), 137.3, 147.0, 155.6, 160.7, 161.8.



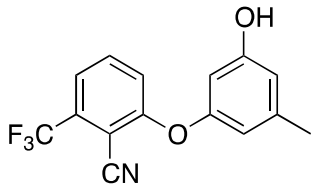
2-(3-(Benzyloxy)-5-hexylphenoxy)-6-(trifluoromethyl)benzonitrile (2.6g)[16]

General Method B. The residue was purified by column chromatography (SiO_2, gradient of hexane) to afford **2.6g** (0.491 g, 98%) as a yellow oil. TLC: R_f 0.49 (EtOAc/hexane, 3:7): ^1H NMR (400 MHz; $CDCl_3$) δ 0.90 (t, J = 5.2 Hz, 3H), 1.31 (s, 6H), 1.59 (td, J = 2.8 Hz, 6.0 Hz, 2H), 2.60 (t, J = 7.6 Hz, 2H), 5.05 (s, 2H), 6.55 (d, J = 1.6 Hz, 2H), 6.75 (s, 1H), 7.07 (d, J = 8.4 Hz, 1H), 7.32-7.45 (m, 6H), 7.55 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.3, 22.8, 29.1, 31.2, 31.9, 36.2, 70.5, 101.0, 104.6(2), 112.9, 113.0, 120.1(2), 120.2, 127.8(2), 128.4, 128.9(2), 134.1(2), 136.7, 147.0, 155.3, 160.4, 161.9.



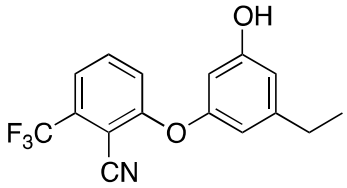
2-(3-Hydroxy-phenoxy)-6-trifluoromethyl-benzonitrile (2.7a)

General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:5, 1:1) to afford **2.7a** (0.199 g, 81%) as a white solid. TLC: R_f 0.08 (EtOAc/hexane, 1:4), mp 106–108 °C: ^1H NMR (400 MHz; $(CD_3)_2CO$) δ 6.77-6.71 (m, 2H), 6.80-6.82 (m, 1H), 7.28-7.34 (m, 2H), 7.66 (d, J = 7.6 Hz, 1H), 7.84-7.88 (m, 1H), 8.81 (broad s, 1, OH). ^{13}C NMR (100 MHz; $(CD_3)_2CO$) δ 101.6, 107.6, 111.1, 112.4, 113.2, 120.8(2), 121.7, 131.3, 135.3(2), 155.9, 159.5, 161.7.



2-(3-Hydroxy-5-methyl-phenoxy)-6-trifluoromethyl-benxonitrile (2.7b)

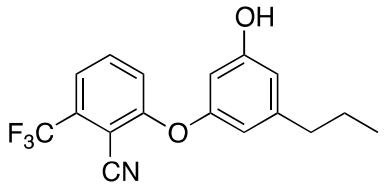
General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:5, 1:1) to afford **2.7b** (0.588 g, 57%) as a light brown solid. TLC: R_f 0.19 (EtOAc/hexane, 1:4). The oily residue was induced to crystallize by the addition of hexane, mp 73-75 °C: ^{1}H NMR (400 MHz; $CDCl_3$) δ 2.29 (s, 3H), 5.79 (broad s, 1, OH), 6.42-6.46 (m, 2H), 6.57 (d, J = 0.8 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 7.6 Hz, 2H), 7.55-7.59 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 20.8, 101.2, 104.7(2), 111.9(2), 113.8, 120.6, 121.2, 135.2(2), 141.8, 155.7, 159.2, 161.8. MS (ESI) m/z 292.1 (M - 1). Anal. Calcd. for $C_{15}H_{10}F_3NO_2$: C, 61.44; H, 3.44; N, 4.78. Found: C, 61.23; H, 3.77; N, 4.54.



2-(3-Ethyl-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (2.7c)

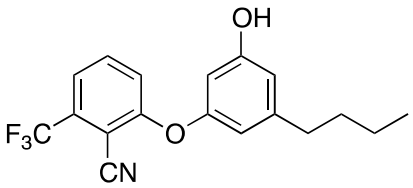
General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:5, 1:3, 1:1) to afford **2.7c** (0.279 g, 89%) as a yellow oil. TLC: R_f 0.37 (EtOAc/hexane, 1:4): ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.21 (p, J = 13.0 Hz, 3H), 2.60(q, J = 12.0 Hz, 2H), 5.32 (s, 1H), 6.43 (t, J = 2.4 Hz, 1H), 6.51 (broad s, 1, OH), 6.60 (t, J = 0.8 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.57 (q, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 15.3, 28.9, 105.3(2), 112.2, 112.7, 120.6, 120.3, 120.2(2), 134.2, 134.3,

148.5, 155.4, 157.4, 161.8. MS (ESI) m/z 342.1 (M + MeOH), 306.1 (M – O^-). Anal. Calcd. for $C_{19}H_{18}F_3NO_2$: C, 62.54; H, 3.94; N, 4.56. Found: C, 62.44; H, 4.07; N, 4.35.



2-(3-Propyl-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (2.7d)

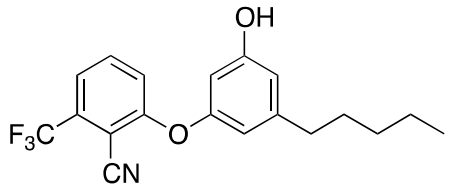
General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:5, 1:3, 1:1) to afford **2.7d** (90.4 mg, 41%) as a yellow oil. TLC: R_f 0.52 (EtOAc/hexane, 3:7): ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.92 (t, J = 7.2 Hz, 3H), 1.61 (q, J = 7.6 Hz, 2H), 2.52 (t, J = 7.6 Hz, 2H), 4.71 (broad s, 1, OH), 6.43 (s, 1H), 6.48 (s, 1H), 6.57 (s, 1H), 7.09 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.56 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 13.9, 24.3, 38.0, 100.2(2), 105.4(2), 112.6, 113.5(2), 120.1, 134.5(2), 146.9, 155.1, 157.6, 162.1. MS (ESI) m/z 364.3 (M + 1), 382.4 (M + H_2O), 386.2 (M + Na^+). Anal. Calcd. for $C_{20}H_{20}F_3NO_2$•1/2 H_2O: C, 64.51; H, 5.68; N, 3.76. Found: C, 64.62; H, 5.62; N, 3.64.



2-(3-Butyl-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (2.7e)

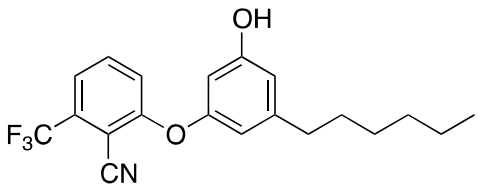
General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:5, 1:3, 1:1) to afford **2.7e** (86.7 mg, 36%) as a yellow

solid. TLC: R_f 0.62 (EtOAc/hexane, 1:4). The oily residue was induced to crystallize by the addition of hexane, mp 89-92 °C: ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.91 (q, J = 7.6 Hz, 3H), 1.30-1.37 (m, 2H), 1.52-1.59 (m, 2H), 2.54 (t, J = 7.2 Hz, 2H), 6.14 (broad s, 1, OH), 6.44 (t, J = 2.4 Hz, 1H), 6.47 (d, J = 2.0 Hz, 1H), 6.59 (dd, J = 1.6 Hz, 1.2 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.54-7.59 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.1, 22.4, 33.3, 35.7, 105.3(2), 112.6, 113.3, 120.1(2), 120.2(2), 134.2(2), 147.2, 155.3, 157.5, 161.9. MS (ESI) m/z 336.1 (M + 1), 353.3 (M + H_2O), 358.1 (M + Na^+). Anal. Calcd. for $C_{18}H_{16}F_3NO_2$: C, 64.47; H, 4.81; N, 4.18. Found: C, 64.71; H, 4.85; N, 4.06.



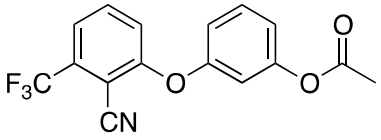
2-(3-Hydroxy-5-pentyl-phenoxy)-6-trifluoromethyl)-benzonitrile (2.7f)

General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:1) to afford **2.7f** (0.141 g, 40%) as a white solid. TLC: R_f 0.23 (EtOAc/hexane, 1:4). The oily residue was induced to crystallize by the addition of hexane, mp 85-86 °C: ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.88 (q, J = 4.4 Hz, 3H), 1.26-1.34 (m, 4H), 1.54-1.62 (m, 2H), 2.54 (t, J = 7.6, 2H), 5.51 (broad s, 1, OH), 6.44 (t, J = 2.0 Hz, 2H), 6.49 (d, J = 2.0 Hz, 1H), 6.58 (s, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.54-7.58 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.2, 22.7, 30.9, 31.6, 35.9, 97.6, 105.2, 112.5, 112.9, 113.1, 115.6(2), 120.2(2), 134.1, 147.3, 155.4, 157.1, 161.8. MS (ESI) m/z 372.2 (M + Na^+). Anal. Calcd. for $C_{19}H_{18}F_3NO_2$: C, 65.32; H, 5.19; N, 4.01. Found: C, 65.50; H, 5.14; N, 4.01.



2-(3-Hexyl-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (2.7g)

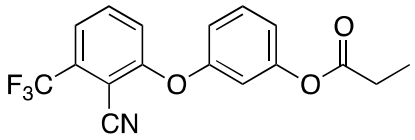
General Method C. The residue was purified by column chromatography (SiO_2, gradient of hexane, EtOAc/hexane, 1:9, 1:7, 1:5, 1:3, 1:1) to afford **2.7g** (33.2 mg, 8.4%) as a yellow solid. TLC: R_f 0.68 (EtOAc/hexane, 3:7). The oily residue was induced to crystallize by the addition of hexane, mp 88-91 °C: ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.86- 0.89 (m, 3H), 1.24-1.33(m, 6H), 1.54-1.60 (m, 2H), 2.54 (t, J = 8.0 Hz, 2H), 5.50 (broad s, 1, OH), 6.43 (t, J = 2.0 Hz, 1H), 6.49 (s, 1H), 6.58 (s, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.56 (t, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.3, 22.8, 29.1, 31.1, 31.8, 36.0, 105.3(2), 112.6(2), 112.8, 113.2, 120.1, 120.2, 134.1(2), 147.3, 155.4, 157.2, 161.8. MS (ESI) m/z 364.3 (M + 1), 382.4 (M + H_2O), 386.2 (M + Na^+). Anal. Calcd. for $C_{20}H_{20}F_3NO_2$•1/2 H_2O: C, 64.51; H, 5.68; N, 3.76. Found: C, 64.62; H, 5.62; N, 3.64.



3-(2-Cyano-3-(trifluoromethyl)phenozy)phenyl acetate (2.8b)

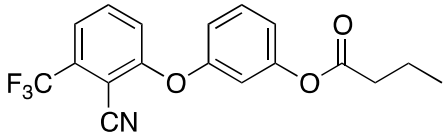
General Method D. The residue was purified by flash chromatography (SiO_2, gradient of hexane, CH_2Cl_2, EtOAc) to afford **2.8b** (46.5 mg, 67%) as a colorless oil. TLC: R_f 0.30 (EtOAc/hexane, 1:4): ^{1}H NMR (400 MHz; $CDCl_3$) δ 2.30 (s, 3H), 6.91 (t, J = 2.0 Hz, 1H), 6.98-7.04 (m, 2H), 7.14 (d, J = 8.8 Hz, 1H), 7.43 (dd, J = 8.0 Hz, 8.8 Hz, 2H), 7.60 (t, J = 7.6 Hz,

1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 21.3, 98.6, 112.3, 114.4, 117.7(2), 119.3, 120.3, 120.7(2), 124.1, 131.0, 134.3(2), 152.1, 155.1, 161.2, 169.2. MS (ESI) m/z 322.3 (M + 1), 344.3 (M + Na^+), 339.3 (M + H_2O). Anal. Calcd. for $C_{16}H_{10}F_3NO_3$•1/4 C_7H_8: C, 61.92; H, 3.51; N, 4.07. Found: C, 60.74; H, 3.66; N, 4.07.



3-(2-Cyano-3-(trifluoromethyl)phenozy)phenyl propionate (2.8c)

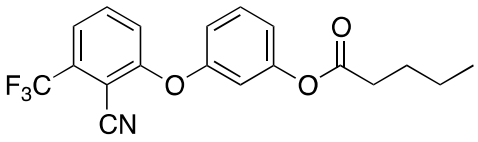
General Method D. The residue was purified by preparative TLC (SiO_2, gradient of EtOAc/hexane 1:4) to afford **2.8c** (70.8 mg, 61%) as a white solid. TLC: R_f 0.25 (EtOAc/hexane, 1:4), mp 82-83 °C: ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.23 (t, J = 7.5 Hz, 3H), 2.59 (q, J = 7.5 Hz, 2H), 6.91 (s, 1H), 6.91-7.04 (m, 2H), 7.13 (d, J = 8.4 Hz, 1H), 7.41-7.49 (m, 2H), 7.60 (t, J = 8.1 Hz, 1H). ^{13}C NMR (300 MHz; $CDCl_3$) δ 9.16, 27.9, 101.4, 112.4, 114.5, 117.6, 119.3, 120.2, 120.6, 124.2, 127.8, 131.0, 134.3, 152.3, 155.0, 161.3, 172.8. MS (ESI) m/z 454.1 (M + 1). Anal. Calcd. for $C_{17}H_{12}F_3NO_3$•1/4 H_2O: C, 60.09; H, 3.71; N, 4.12. Found: C, 60.20; H, 3.49; N, 4.16.



3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl butyrate (2.8d)

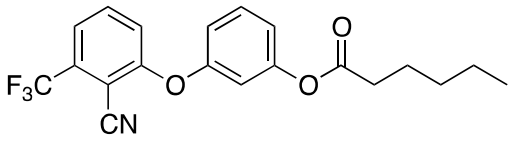
General Method D. The residue was purified by preparative TLC (SiO_2, gradient of EtOAc/hexane 1:4) to afford **2.8d** (0.128 g, 69%) as a pale yellow oil. TLC: R_f 0.54 (EtOAc/

CH_2Cl_2, 1:4): ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.03 (t, J = 7.6 Hz, 3H), 1.77 (sextet, J = 7.6 Hz, 2H), 2.54 (t, J = 7.6 Hz, 2H), 6.90 (t, J = 2.4 Hz, 1H), 6.97-7.04 (m, 2H), 7.13 (d, J = 8.4 Hz, 1H), 7.42-7.48 (m, 2H), 7.57-7.62 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 13.8, 18.6, 36.4, 101.7, 112.4, 114.5, 117.6, 119.3, 120.2, 120.5, 120.6, 131.0, 134.3(2), 152.2, 155.0, 161.3, 172.0. MS (ESI) m/z 350.3 (M + 1), 372.3 (M + Na^+), 367.4 (M + H_2O). Anal. Calcd. for $C_{20}H_{20}F_3NO_2$•1/2 H_2O: C, 64.51; H, 5.68; N, 3.76. Found: C, 64.62; H, 5.62; N, 3.64.



3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl pentanoate (2.8e)

To a stirred solution of phenol (23a) (57 mg, 0.20 mmol) in Et_3N (5 mL) valeryl chloride (29 µL, 0.24 mmol, 1.2 equiv) was added and refluxed for 2 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, gradient of hexane, hexane/ CH_2Cl_2 1:1, CH_2Cl_2, $(CH_3)_2CO$) to afford **2.8e** (57.1 mg, 78%) as a white solid. TLC: R_f 0.50 (EtOAc/hexane, 1:4), mp 44-46 °C: ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.96 (t, J = 7.2 Hz, 3H), 1.39-1.48 (m, 2H), 1.69-1.76 (m, 2H), 2.56 (t, J = 7.2 Hz, 2H), 6.90 (t, J = 2.4 Hz, 1H), 6.97-7.03 (m, 2H), 7.13 (d, J = 8.8 Hz, 1H), 7.41-7.48 (m, 2H), 7.57-7.61 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 13.9, 22.4, 27.1, 34.2, 101.3, 112.4, 114.5, 117.7, 119.4, 120.2, 120.6, 120.7, 123.7, 131.0, 134.3, 152.3, 155.0, 161.3, 172.2. MS (ESI) m/z 454.1 (M + 1). Anal. Calcd. for $C_{20}H_{20}F_3NO_2$•1/2 H_2O: C, 64.51; H, 5.68; N, 3.76. Found: C, 64.62; H, 5.62; N, 3.64.



3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl hexanoate (2.8f)

General Method D. The residue was purified by preparative TLC (SiO_2, gradient of EtOAc:hexane 1:4) to afford **2.8f** (58.0 mg, 74%) as a colorless oil. TLC: R_f 0.56 (EtOAc/hexane, 1:4): ^1H NMR (400 MHz; $CDCl_3$) δ 0.91-0.94 (m, 3H), 1.36-1.40 (m, 4H), 1.71-1.76 (m, 2H), 2.55 (t, J = 7.2 Hz, 2H), 6.90 (t, J = 2.4 Hz, 1H), 6.97-7.03 (m, 2H), 7.13 (d, J = 8.4 Hz, 1H), 7.42-7.48 (m, 2H), 7.59 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.1, 22.5, 24.7, 31.4, 34.4, 112.3, 114.5, 117.6, 119.3, 120.2, 120.6(2), 120.7, 123.7, 131.0, 134.3, 152.2, 155.0, 161.3, 172.1. MS (ESI) m/z 378.3 (M + 1), 400.4 (M + Na^+). Anal. Calcd. for $C_{20}H_{18}F_3NO_3$: C, 63.66; H, 4.81; N, 3.71. Found: C, 63.84; H, 4.90; N, 3.65.

2.7 References

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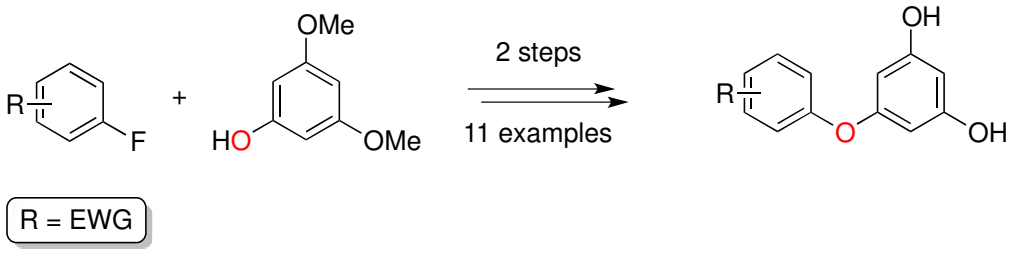
3

General Method for the Synthesis of Phloroglucinol Monoaryl Ethers Towards the Development of CB1 Receptor Ligands

3.1 Abstract

A novel 1,3,5-trihydroxybenzene core diaryl ether scaffold was developed in order to establish a library of precursor compounds for a cannabinoid structure activity relationship study. A variety of novel functionalized phloroglucinol monoaryl ethers have been synthesized in two steps under mild conditions by coupling substituted aryl fluorides with 3,5-dimethoxyphenol followed by conversion of the methyl ethers to phenols. Target compounds were synthesized under simple reaction conditions via nucleophilic aromatic substitution (S_NAr) in N-methylpyrrolidine and cesium carbonate. Subsequent boron tribromide mediated demethylation gave a series of monoaryl ethers in good overall yields. The compounds presented here represent a relatively unexplored substructure of molecules and may be used as substrates in exploring a variety of chemical applications.

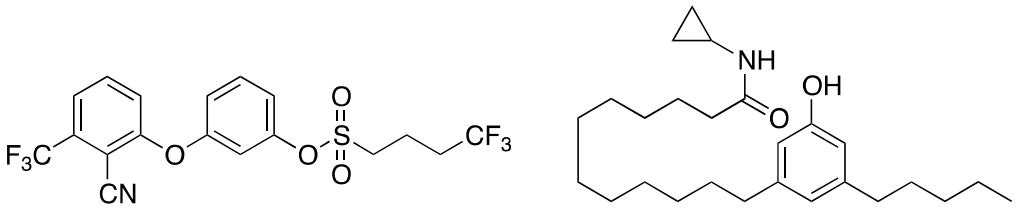
Scheme 3.1 Abstract scheme, the synthesis of phloroglucinol monoaryl ethers



3.2 Introduction

In our efforts to synthesize novel analogues of the cannabinoid partial agonists BAY 59-3074 and CB-25 **(Figure 3.1)**, we have developed a simple, catalyst-free procedure for the synthesis of phloroglucinol monoaryl ethers. To our knowledge, no formal report exists describing the synthesis of phloroglucinol monoaryl ethers. The structures presented here represent a relatively unexplored substructure of compounds that were synthesized specifically for evaluation of the cannabinoid pharmacophore, but may also find utility elsewhere.

Figure 3.1. Cannabinoid partial agonists BAY 59-3074 and CB-25

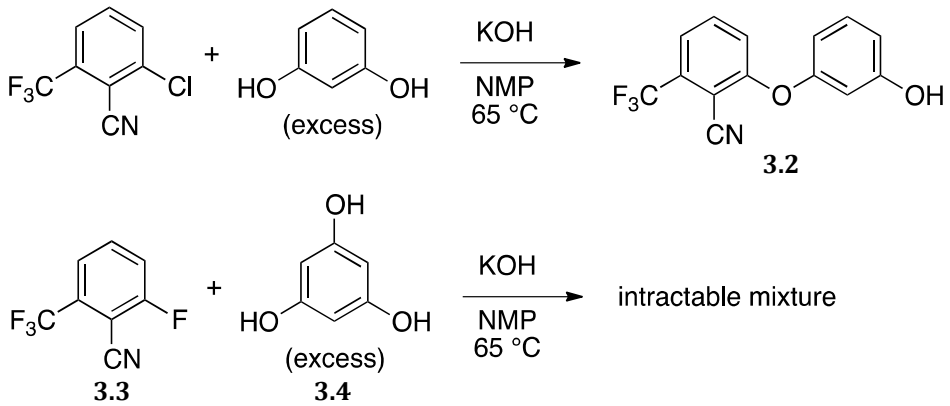


2.2 **2.3**

Several methods have been reported for the preparation of diaryl ethers, many of which rely on copper catalysts.[1,2] We have chosen to proceed through uncatalyzed nuclephilic aromatic substitution (S_NAr) promoting conditions for the sake of simplicity.

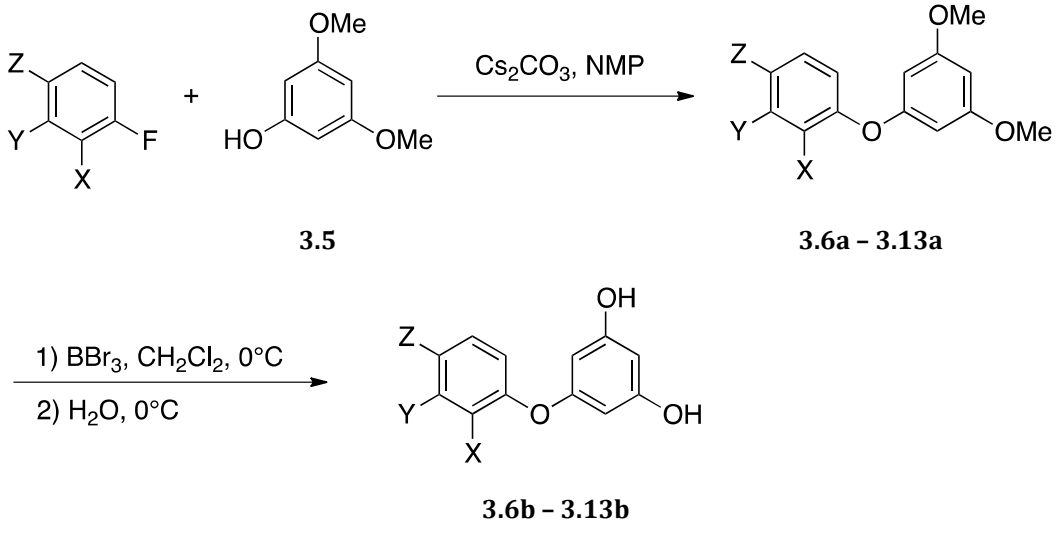
Reactions that proceed via an S_NAr mechanism represent one of the more attractive methods for diaryl ether synthesis due to mild reaction conditions typically employed to affect the desired coupling.[3,4] The preparation of the diaryl ether precursor **3.2** to BAY 59-3074 was achieved via uncatalyzed conditions in good yield in N-methylpyrrolidone (NMP) using KOH as a base **(Scheme 3.2)**.[5] Other reports have also demonstrated NMP to be advantageous in the synthesis of diaryl ethers.[6] As a solvent, NMP is ideal for these uncatalyzed coupling reactions since it possesses a high boiling point (203 °C) and large dielectric constant (32.2). Based upon these results we initially explored the uncatalyzed conditions developed for the synthesis of **3.2** for the aryl ether coupling reaction of aryl fluoride **3.3** with phloroglucinol **(3.4)**. Unfortunately, the direct arylation of **3.4** proved to be less straightforward. The uncatalyzed conditions led to a complex mixture of mono- and diaryl ethers as well as an intractable mixture of C-arylation products **(Scheme 3.3)**. In addition, the removal of the excess phloroglucinol made the isolation and purification of the coupling products cumbersome and tedious.

Scheme 3.2 Attempted direct arylation of phloroglucinol



In light of these results, we sought to develop a stoichiometric uncatalyzed method for the preparation of the desired phloroglucinol monoaryl ethers. To this end, a two-step process was envisaged that would involve initial aryl ether formation via coupling a substituted aryl fluoride with a protected phloroglucinol derivative **3.5** followed by a deprotection step to give the phloroglucinol monoaryl ether **(Scheme 3.3)**. 3,5-Dimethoxyphenol **(3.5)** seemed to be a particularly amenable substrate for the uncatalyzed coupling reaction due to electron-donating methoxy groups that would activate the phenol toward S_NAr nucleophilic addition. The methyl ethers could then be readily removed to furnish the phloroglucinol derivative. Cesium carbonate was selected as the base for the reaction since it has been widely used for the generation of phenoxide salts in diaryl ether coupling reactions due to its functional group tolerance and good solubility in organic solvents.[7–10]

Scheme 3.3 Two-step synthesis of phloroglucinol monoaryl ethers



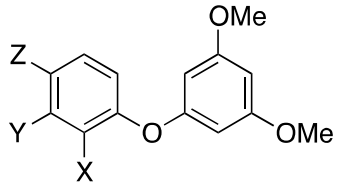
3.5 **3.6a – 3.13a**

3.6b – 3.13b

3.3 Results and Discussion

As summarized in **Table 3.1** the uncatalyzed coupling reactions of substituted aryl fluorides with **3.5** gave the corresponding diaryl ethers **3.6a – 3.13a** in good to high yields. The coupling reactions presented here support an S_NAr mechanism. Substrates that possessed strong activating groups such as nitrile (**3.6a** and **3.12a**) and nitro (**3.13a**) gave nearly quantitative yields, presumably via formation of an intermediate Meisenheimer complex.[11] However, weaker activating groups like halogen (Cl, Br, I) also gave good yields when multiple halogen substituents were present. Only aryl fluorides that did not contain electron withdrawing groups or were mono-halogenated (**3.14a** - **3.16a**) were unreactive.[12]

Table 3.1 Uncatalyzed arylation of 3,5-dimethoxyphenol (3.5)



3.6a - 3.13a

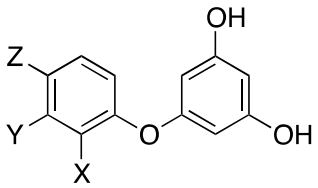
Cpd	Code	X	Y	Z	Product (yield, %)
3.6a	AMS115	CN	CF_3	H	99
3.7a	AMS191	Cl	H	Cl	84
3.8a	AMS209	Cl	Cl	H	59
3.9a	AMS211	H	Cl	Cl	60
3.10a	AMS235	Br	H	Br	51[a]
3.11a	DP173	I	H	Cl	62
3.12a	DP171	H	H	CN	94
3.13a	AMS123	H	H	NO_2	94
3.14a	AMS213a	H	Cl	H	(NR)[b]
3.15a	AMS213b	H	H	H	(NR)[b]
3.16a	AMS213c	H	H	Me	(NR)[b]

[a] Mixture of isomers. [b] NR: no reaction, starting material recovered

The coupling to m-chlorofluorobenzene was not at all reactive **(3.14a)**, yet reactions of fluorobenzenes bearing dichloro substitution **(3.7a – 3.9a)** and dibromo substitution **(3.10a**) proceeded in good yields. Neither fluorotoluene nor fluorobenzene were reactive under the conditions presented **(3.15a**, **3.16a)**. Additionally, clear selectivity was demonstrated for nucleophilic attack at the fluorine-substituted carbon over other halogenated sites. In all examples, including where mixed aryl halides were employed **(3.11a)**, the predominant product was always formed chemoselectively by substitution at the fluorinated carbon.

The dimethoxy diaryl ethers **3.6a** – **3.13a** were converted into the phloroglucinol derivatives **3.6b** – **3.13b** using boron tribromide. This demethylation process has been well covered in the literature.[13,14] However, it is worth noting that special care must be taken with the quench/hydrolysis procedure in order to achieve high yields. It was found that slowly dripping the reaction mixture into an excess of stirred cold water produced the best results. All of the reactions proceeded cleanly and in high yield (>89%) with the exception of the nitrile **3.6a**. The demethylation step gave a mixture of side products that made it necessary to purify the phloroglucinol derivative **(Table 3.2, 3.6b)** by column chromatography. All other compounds were isolated in sufficient purity for subsequent use directly from the workup with no chromatography.

Table 3.2 Monoaryl phloroglucinol derivatives



3.6b – 3.13b

Cpd	Code	X	Y	Z	Product (yield, %)
3.6b	AMS149	CN	CF_3	H	82
3.7b	AMS193	Cl	H	Cl	97
3.8b	AMS217	Cl	Cl	H	97
3.9b	AMS219	H	Cl	Cl	97
3.10b	AMS239	Br	H	Br	89[a]
3.11b	AMS241	I	H	Cl	95
3.12b	AMS223	H	H	CN	98
3.13b	AMS221	H	H	NO_2	92

[a] Mixture of isomers.

3.4 Conclusion

In conclusion, an efficient, safe, and scalable method for the preparation of phloroglucinol monoaryl ethers has been developed. The uncatalyzed aryl coupling reaction was chemoselective and furnished the diaryl ethers cleanly with few side reactions. Further manipulation of this unique molecular scaffold toward the development of novel cannabinoid receptor ligands will be reported elsewhere.

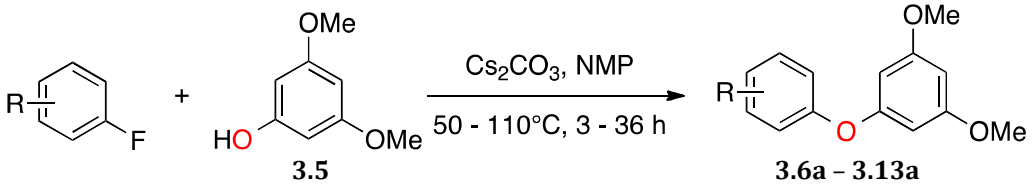
3.5 Acknowledgement

This research was funded by the National Institute on Drug Abuse (DA023916) and the University of New Orleans. David M. Pond assisted in the work presented here as part of his CHEM 3027 requirements.

3.6 Experimental Section

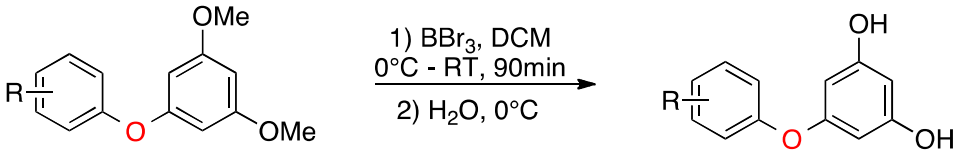
General methods

All chemicals were purchased from Aldrich Chemical Company and used as received unless otherwise noted. TLC: silica gel (250 μm); visualization with UV light, I_2, or phosphomolybdic acid. Chromatography: silica gel 60 Å (230–400 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian 400 MHz NMR spectrometer at ambient temperature in $CDCl_3$ or DMSO-d_6. ^{1}H NMR chemical shifts are reported as δ values (ppm) relative to TMS. ^{13}C NMR chemical shifts are reported as δ values (ppm) relative to $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.5 ppm). Melting points were recorded on a Mel-temp apparatus and are uncorrected. Atlantic Microlab, Inc., Norcross, GA performed all CHN microanalyses.



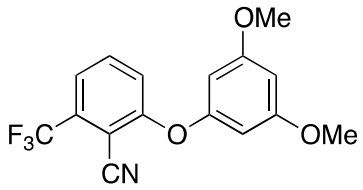
General Method A. (diaryl ether formation). To 3,5-dimethoxyphenol (1.1 equiv) dissolved in NMP (0.7-1.4 mL/mmol) was added Cs_2CO_3 (3 equiv). A rubber septum was attached to the reaction flask and a nitrogen atmosphere was established by evacuation and back filling with nitrogen, repeated three times. The flask was placed in a 50°C oil bath for 30 minutes resulting in a dark brown phenoxide solution. Aryl fluoride (1 equiv.) was syringed into the solution and the reaction was allowed to stir at 50-110 °C for 3-36h. Reaction temperature was adjusted to not exceed the boiling point aryl fluoride used. Reactions were monitored by TLC to determine apparent completion. The reaction

mixtures were cooled to room temperature and added to H_2O (20 mL). The resulting suspension was extracted with toluene (3 x 15 mL). The pooled organic extracts were washed with H_2O (15 mL), brine (15 mL) then dried over $MgSO_4$ and filtered. The toluene was distilled off under reduced pressure on a rotoevaporator. The resulting residue was purified by column chromatography or triturated with H_2O (10 mL), filtered, and washed with water then dried. Compounds isolated by trituration were of adequate purity to be used for further synthesis.



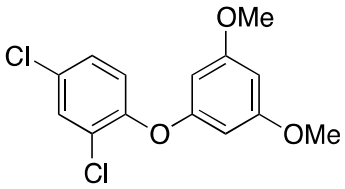
3.6a – 3.13a **3.6b – 3.13b**

General Method B. (BBr_3 promoted demethylation) A solution of dimethoxy diaryl ether **3.6a – 3.13a** (1 equiv.) was dissolved in anhydrous CH_2Cl_2 (5 mL/mmol) and stirred under N_2 at 0 °C for 15 min. With vigorous stirring, BBr_3 (3–5 equiv) was carefully syringed into the solution over 15 min. The ice bath was removed and the mixture was stirred for 90 min at r.t. The reaction mixture was carefully transferred to an addition funnel and added dropwise to H_2O (50 mL) at 0 °C with continuous stirring over 20 min. The resulting suspension was extracted with EtOAc (4 × 40 mL). The combined organic extracts were washed with H_2O (50 mL), brine (50 mL), then dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure to afford a viscous oil. The oily residue was lyophilized under high vacuum to afford the phloroglucinol aryl ethers **3.6b-3.13b** as solids in pure form.



2-(3,5-dimethoxyphenoxy)-6-(trifluoromethyl)benzonitrile (3.6a)

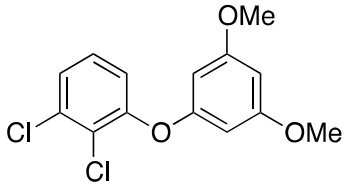
General Method A. 3,5-dimethoxyphenol (3.4 g, 22 mmol) was reacted with Cs_2CO_3 (9.8 g, 30 mmol) and 2-fluoro-6-(trifluoromethyl)benzonitrile (3.7 g, 20 mmol) in NMP (15 mL) at 65 °C for 4 h. Purification by trituration and filtration afforded **3.6a** (6.4 g, 99 %) as a shiny white solid; mp 95-97 °C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 7.57 (t, J = 8.0 Hz, 1H) 7.44 (d, J = 8.0Hz, 1H) 7.13 (d, J = 8 Hz, 1H) 6.36 (dd, J = 2.0, 2.0Hz, 1H), 6.25(t, J = 2.0Hz, 2H), 3.78 (s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.2, 161.5, 156.1, 134.4, 134.1, 123.8, 121.1, 120.4, 112.6, 100.8, 98.9, 97.9, 55.7.



2,4-dichloro-1-(3,5-dimethoxyphenoxy)benzene (3.7a).

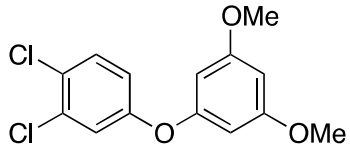
General Method A. 3,5-dimethoxyphenol (3.4 g, 22 mmol) was reacted with Cs_2CO_3 (9.8 g, 30 mmol) and 2,4-dichloro-1-fluorobenzene (3.3 g, 20 mmol) in NMP (15 mL) at 120 °C for 2 h. Purification by flash chromatography (10% ethyl acetate/hexane) afforded **3.7a** (5.0 g, 84 %) as a white solid; mp 44-45°C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 7.48 (d, J = 2.0 Hz, 1H) 7.19(dd, J = 8.0, 2.0 Hz, 1H) 6.96 (d, J = 8.0Hz, 1H) 6.22(dd, J = 2.0, 2.0 Hz, 1H) 6.10(t, J = 2.0

Hz 2H) 3.74 (s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.9, 158.7, 151.2, 130.7, 129.7, 128.3, 127.0, 122.1, 96.7, 96.0, 55.7.



1,2-dichloro-3-(3,5-dimethoxyphenoxy)benzene (3.8a).

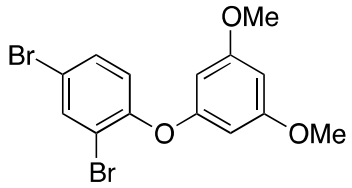
General Method A. 3,5-dimethoxyphenol (3.4 g, 22 mmol) was reacted with Cs_2CO_3 (9.8 g, 30 mmol) and 1,2-dichloro-3-fluorobenzene (3.3 g, 20 mmol) in NMP (20 mL) at 110 °C for 20 h. Purification by flash chromatography (10% ethyl acetate/hexane) afforded **3.8a** (3.5 g, 59%) as a shiny white solid; mp 43-44 °C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 7.26 (dd, J = 8.0, 2.0 Hz, 1H) 7.15 (t, J = 8.0 Hz, 1H) 6.94 (dd, J = 8.0, 2.0 Hz 1H) 6.24 (dd, J = 2.0, 2.0 Hz, 1H) 6.13(d, J = 2.0 Hz, 2H) 3.76 (s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.0, 158.6, 154.9, 134.2, 127.8, 125.7, 125.2, 119.0, 97.0, 96.1, 55.7.



1,2-dichloro-4-(3,5-dimethoxyphenoxy)benzene (3.9a).

General Method A. 3,5-dimethoxyphenol (1.9 g, 12 mmol) was reacted with Cs_2CO_3 (5.4 g, 17 mmol) and 1,2-dichloro-4-fluorobenzene (1.9 g, 11 mmol) in NMP (10 mL) at 120 °C for 2 h. Purification by flash chromatography (10% ethyl acetate/hexane) afforded **3.9a** (2.0 g, 60%) as a white solid; mp 57-58°C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 7.37 (d, J = 8.0 Hz, 1H) 7.12 (d, J = 2.0 Hz, 1H) 6.88 (dd, J = 8.0, 2.0 Hz, 1H) 6.27 (dd, J = 2.0, 2.0 Hz, 1H) 6.17 (t, J = 2.0 Hz,

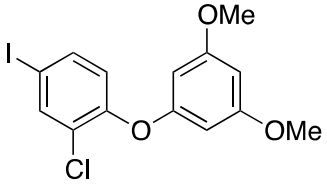
2H) 3.76(s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.0, 158.2, 156.5, 131.2, 120.8, 118.5, 115.6, 98.0, 96.6, 55.7.



2,4-dibromo-1-(3,5-dimethoxyphenoxy)benzene (3.10a).

General Method A. 3,5-dimethoxyphenol (1.1 g, 7.3 mmol) was reacted with Cs_2CO_3 (3.3 g, 10 mmol) and 2,4-dibromo-1-fluorobenzene (1.7 g, 6.7 mmol, 90% purity*) in NMP (10 mL) at 65 °C for 36 h. Purification by flash chromatography (10% ethyl acetate/hexane) afforded **3.10a** (1.2 g, 51%) as a mixture of isomers* as a clear oil: ^1H NMR (400MHz; $CDCl_3$) δ: 7.76 (d, J = 2.4 Hz, 1H) 7.37(dd, J = 8.0, 2.4 Hz, 1H) 6.88 (d, J = 8.0Hz, 1H) 6.24(t, J = 4.0 Hz, 1H) 6.12(d, J = 2.0 Hz, 2H) 3.74 (s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.0, 158.5, 153.0, 136.2, 131.9, 122.1 117.0, 116.0, 97.0, 96.1, 55.7.

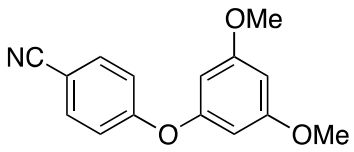
*starting material contained a 10% mixture of dibromofluorobenzene isomers



2-chloro-1-(3,5-dimethoxyphenoxy)-4-iodobenzene (3.11a).

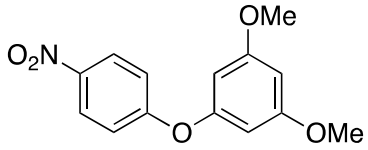
General Method A. 3,5-dimethoxyphenol (0.66 g, 4.3 mmol) was reacted with Cs_2CO_3 (1.7 g, 5.1 mmol) and 2-chloro-1-fluoro-4-iodobenzene (1.1 g, 4.2 mmol) in NMP (10 mL) at 68 °C for 24 h. Purification by trituration and filtration afforded **3.11a** (1.0 g, 62%) as a tan-

red solid; mp 72-74°C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 7.77 (d, J = 2.0 Hz, 1H) 7.50(dd, J = 8.0, 2.0 Hz, 1H) 6.76 (d, J = 8.0Hz, 1H) 6.24(dd, J = 2.0, 2.0 Hz, 1H) 6.13(d, J = 2.0 Hz, 2H) 3.74 (s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.0, 158.4, 153.7, 139.1, 137.2, 122.7, 97.0, 96.2, 55.7.



4-(3,5-dimethoxyphenoxy)benzonitrile (3.12a).

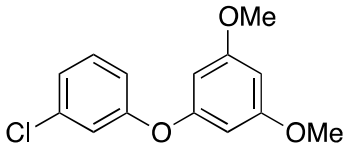
General Method A. 3,5-dimethoxyphenol (1.1 g, 7.3 mmol) was reacted with Cs_2CO_3 (3.3 g, 10 mmol) and 4-fluorobenzonitrile (0.81 g, 6.7 mmol) in NMP (10 mL) at 60°C for 48h. Purification by trituration and filtration afforded **3.12a** (1.6 g, 94%) as a light orange solid; mp 67-68°C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 7.60 (d, J = 8.0 Hz, 2H) 7.03 (d, J = 8.0 Hz, 2H) 6.32 (t, J = 2.0 Hz, 1H) 6.21 (d, J = 2.0 Hz, 2H) 3.76(s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.1, 161.5, 156.8, 134.8, 119.0, 118.4, 106.8, 99.0, 97.4, 55.7.



1,3-dimethoxy-5-(4-nitrophenoxy)benzene (3.13a).

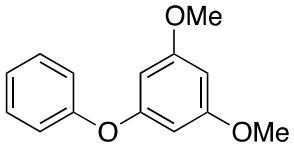
General Method A. 3,5-dimethoxyphenol (1.1 g, 7.3 mmol) was reacted with Cs_2CO_3 (3.3 g, 10 mmol) and 1-fluoro-4-nitrobenzene (0.95 g, 6.7 mmol) in NMP (10 mL) at 65 °C for 2 h. Purification by trituration and filtration afforded **3.13a** (1.75 g, 94%) as a light yellow solid; mp 116-118°C: ^{1}H NMR (400MHz; $CDCl_3$) δ: 8.20 (d, J = 8.0 Hz, 2H) 7.05 (d, J = 8.0 Hz,

2H) 6.34 (t, J = 4.0 Hz, 1H) 6.24 (d, J = 2.0 Hz, 2H) 3.78(s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 163.3, 162.2, 156.7, 126.1, 117.5, 115.6, 99.6, 97.7, 55.8.



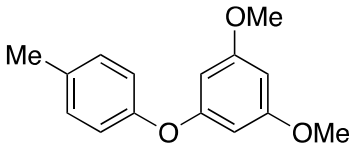
1-(3-chlorophenoxy)-3,5-dimethoxybenzene (3.14a).

General Method A. 3,5-dimethoxyphenol (1.1 g, 7.3 mmol) was reacted with Cs_2CO_3 (3.3 g, 10 mmol) and 1-chloro-3-Fluorobenzene (0.86 g, 6.7 mmol) was added in NMP (10 mL) at 62 ^{0}C for 23 h. No product formation was observed. The reaction was repeated in a sealed tube reactor, but again no product formation was evident.



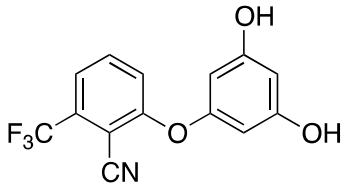
(1,3-dimethoxy-5-phenoxybenzene (3.15a).

General Method A. 3,5-dimethoxyphenol (0.67 g, 4.3 mmol) was reacted with Cs_2CO_3 (1.7 g, 5.1 mmol) and fluorobenzene (0.38 g, 3.9 mmol) was added in NMP (10 mL) at 55 ^{0}C for 24 h. No product formation was observed.



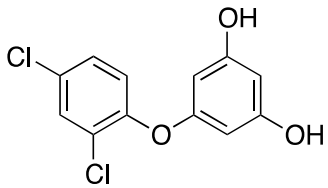
(1,3-dimethoxy-5-(*p*-tolyloxy)benzene (3.16a).

General Method A. 3,5-dimethoxyphenol (0.67 g, 4.3 mmol) was reacted with Cs_2CO_3 (1.7 g, 5.1 mmol) and fluorotoluene (0.43 g, 3.9 mmol) was added in NMP (10 mL) at 65 ^{0}C for 24 h. No product formation was observed.



2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (3.6b).

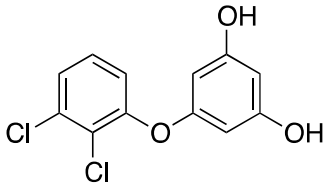
General Method B. To a stirring solution of **3.6a** (4.0 g, 12 mmol) in methylene chloride (60 mL) at 0 °C was carefully added BBr_3 (5.9 mL, 62 mmol). The resulting oily residue was purified by flash chromatography (50% ethyl acetate/hexane) affording **3.6b** (3.0 g, 82%) as a white solid; mp: 178–180 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.66 (s, 2H) 7.81(t, J = 8.0 Hz, 1H) 7.62(d, J = 8.0 Hz, 1H) 7.31(d, J = 8.0 Hz, 1H) 6.17(s, 1H) 6.00(s, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 161.5, 160.5, 156.5, 136.2, 132.7, 124.3, 122.3, 121.4, 113,2, 100.7, 100.0, 98.7. Anal. Calcd for $C_{14}H_8F_3NO_3$: C, 56.96; H, 2.73; N, 4.74. Found: C, 56.90; H, 2.83; N, 4.54.



5-(2,4-dichlorophenoxy)benzene-1,3-diol (3.7b).

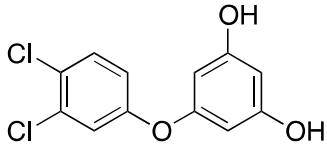
General Method B. To a stirring solution of **3.7a** (0.19 g, 0.64 mmol) in methylene chloride (3.5 mL) at 0 °C was carefully added BBr_3 (0.32 mL, 3.4 mmol). The resulting oily residue

was frozen with liquid nitrogen then subjected to vacuum affording **3.7b** (0.17 g, 97%) as a tan solid; mp: 79–81 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.45 (br s, 2H) 7.71 (d, J = 2.0 Hz, 1H) 7.40(dd, J = 8.0, 2.0 Hz, 1H) 7.11 (d, J = 8.0 Hz, 1H) 5.98(dd, J = 8.0, 2.0 Hz, 1H) 5.76(d, J = 2.0 Hz, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 160.1, 158.7, 151.3, 130.7, 129.4, 129.2, 126.6, 123.5, 98.8, 96.5. Anal. Calcd for $C_{12}H_8Cl_2O_3$: C, 53.17; H, 2.97. Found: C, 53.21; H, 2.80.



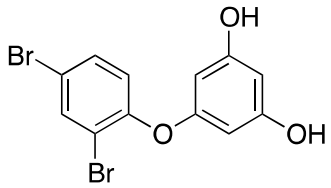
5-(2,3-dichlorophenoxy)benzene-1,3-diol (3.8b).

General Method B. To a stirring solution of **3.8a** (0.19 g, 0.64 mmol) in methylene chloride (3.5 mL) at 0 °C was carefully added BBr_3 (0.32 mL, 3.4 mmol). The resulting oily residue was frozen with liquid nitrogen then subjected to vacuum affording **3.8b** (0.17 g, 97%) as an off-white solid; mp: 104–106 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.46 (s, 2H) 7.45(dd, J = 4.0, 2.0 Hz, 1H) 7.36(t, J = 8.0 Hz, 1H) 7.08 (dd, J = 8.0, 2.2 Hz, 1H) 5.98(dd, J = 2.0, 2.0 Hz, 1H) 5.77(d, J = 2.0 Hz, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 160.2, 158.5, 153.8, 133.4, 129.6, 126.4, 124.4, 120.5, 99.0, 96.6. Anal. Calcd for $C_{12}H_8Cl_2O_3$: C, 53.17; H, 2.97. Found: C, 53.01; H, 3.00.



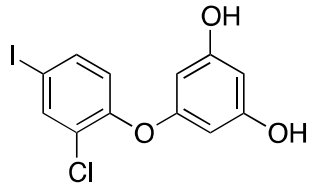
5-(3,4-dichlorophenoxy)benzene-1,3-diol (3.9b).

General Method B. To a stirring solution of **3.9a** (0.19 g, 0.64 mmol) in methylene chloride (3.5 mL) at 0 °C was carefully added BBr_3 (0.32 mL, 3.4 mmol). The resulting oily residue was frozen with liquid nitrogen then subjected to vacuum which afforded **3.9b** (0.16 g, 92%) as an off-white solid; mp: 91 – 92 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.49 (br s, 2H) 7.59(dd, J = 8.0, 2.0 Hz, 1H) 7.26(d, J = 2.0 Hz, 1H) 6.99 (dd, J = 8.0, 2.0 Hz, 1H) 6.02(br s, 1H) 5.85(d, J = 2.0 Hz, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 160.2, 158.0, 156.9, 132.5, 132.1, 125.9, 121.0, 119.6, 99.5, 98.0. Anal. Calcd for $C_{12}H_8Cl_2O_3$: C, 53.17; H, 2.97. Found: C, 53.17; H, 3.17.



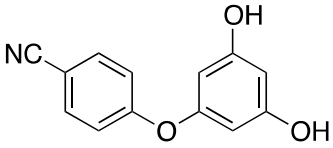
5-(2,4-dibromophenoxy)benzene-1,3-diol (3.10b).

General Method B. To a stirring solution of **3.10a** (0.4 g, 1.0 mmol) in methylene chloride (5.0 mL) at 0 °C was carefully added BBr_3 (0.5 mL, 5.3 mmol). The resulting oily tan residue was frozen with liquid nitrogen and subjected to vacuum, which afforded **3.10b** (0.33 g, 89%) as a red semi-solid: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.45 (s, 2H) 7.94(d, J = 2.0 Hz, 1H) 7.66(dd, J = 8.0, 2.0 Hz, 1H) 7.02 (d, J = 8.0 Hz, 1H) 5.97(t, J = 2.0 Hz, 1H) 5.75(d J = 2.0 Hz, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 160.1, 158.6, 153.0, 136.1, 132.9, 123.6, 117.1, 116.3, 98.8, 96.6. Anal. Calcd for $C_{12}H_8Br_2O_3$: C, 40.04; H, 2.24. Found: C, 39.71; H, 2.44.



5-(2-chloro-4-iodophenoxy)benzene-1,3-diol (3.11b).

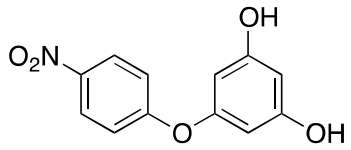
General Method B. To a stirring solution of **3.11a** (0.25 g, 0.64 mmol) in methylene chloride (3.5 mL) at 0 °C was carefully added BBr_3 (0.32 mL, 3.4 mmol). The resulting oily tan residue was frozen with liquid nitrogen and subjected to vacuum which afforded **3.11b** (0.22 g, 95%) as a brown solid; mp: 75 – 77 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.44 (s, 2H) 7.90(d, J = 2.0 Hz, 1H) 7.66(dd, J = 8.0, 2.0 Hz, 1H) 6.88 (d, J = 8.0 Hz, 1H) 5.98(t, J = 2.0 Hz, 1H) 5.76(d, J = 2.0 Hz, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 160.1, 158.5, 152.3, 138.8, 138.1, 126.8, 124.0, 98.9, 96.6, 88.7. Anal. Calcd for $C_{12}H_8ClIO_3$: C, 39.75; H, 2.22. Found: C, 39.50; H, 2.50.



4-(3,5-dihydroxyphenoxy)benzonitrile (3.12b).

General Method B. To a stirring solution of **3.12a** (0.70 g, 2.7 mmol) in methylene chloride (15 mL) at 0 °C was carefully added mmol BBr_3 (1.3 mL, 13 mmol). The resulting oily residue was frozen with liquid nitrogen then subjected to vacuum, which afforded **3.12b** (0.6 g, 98%) as an orange solid; mp: 158–160 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.57 (br s, 2H) 7.79(d, J = 8.0 Hz, 2H) 7.08(d, J = 8.0 Hz, 2H) 6.10(dd, J = 2.0, 2.0 Hz 1H) 5.92(d, J = 2.0 Hz, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 161.6, 160.3, 156.8, 135.2, 119.4, 118.3, 105.6,

100.1, 98.9. Anal. Calcd for $C_{12}H_8Cl_2O_3$: C, 68.67; H, 3.99; N, 6.16. Found: C, 68.51; H, 4.11; N, 6.02.



5-(4-nitrophenoxy)benzene-1,3-diol (3.13b).

General Method B. To a stirring solution of **3.13a** (2.0 g, 7.7 mmol) in methylene chloride (30 mL) at 0 °C was carefully added BBr_3 (2.5 mL, 27 mmol). The resulting oily residue was frozen with liquid nitrogen then subjected to vacuum, which afforded **3.13b** (1.6 g, 89%) as a light yellow solid; mp: 127–129 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.62 (br s, 2H) 8.22 (d, J = 8.0 Hz, 2H) 7.11(d, J = 8.0 Hz, 2H) 6.12(br s, 1H) 5.94(br s, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 163.5, 160.4, 156.6, 142.8, 126.7, 118.1, 100.4, 99.2. Anal. Calcd for $C_{12}H_8Cl_2O_3$: C, 58.30; H, 3.67; N, 5.67. Found: C, 58.81; H, 3.99; N, 5.29.

3.7 References

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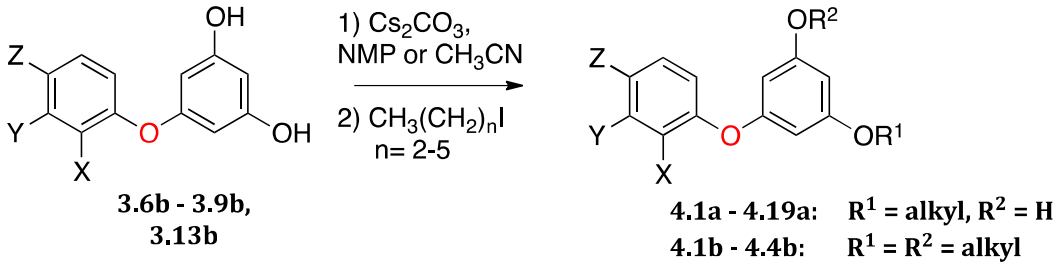
4

Modification of Phloroglucinol Monoaryl Ethers Towards the Development of CB_1 Receptor Ligands

4.1 Abstract

Guided by preliminary data on the utility of alkyl diaryl ethers as potential cannabinoid receptor ligands, it was hypothesized that novel phloroglucinol monoaryl ether core compounds would show binding affinity at the CB1 receptor. Phloroglucinol monoaryl ethers described in Chapter 3 were transformed to the corresponding mixture of mono and di-alkyl ethers in one synthetic step utilizing uncatalyzed Williamson ether synthesis conditions in ether N-methylpyrrolidine or acetonitrile. Though the dialkyl ethers were not our target compounds, some were isolated and characterized and made available for bioassay in the interest of expanding the structure activity relationship studies. Some attempts were made to optimize starting conditions in order to favor the production of mono-alkylated product, but were not particularly successful due to the complex kinetics of the reaction. The compounds presented here were ultimately subjected to biological assessment to determine binding affinity at the cannabinoid receptor in order to verify the aforementioned hypothesis.

Scheme 4.1 Abstract scheme, alkylation of phloroglucinol monoaryl ethers



3.6b - 3.9b, 3.13b

4.1a - 4.19a: R^1 = alkyl, R^2 = H
4.1b - 4.4b: $R^1 = R^2$ = alkyl

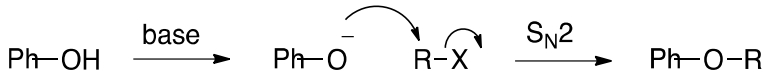
4.2 Introduction

The compounds described herein were synthesized with the goal of further demonstrating that modification of a diaryl ether scaffold would have an effect on binding affinity at the CB1 receptor. Guided in part by the preliminary efforts to synthesize novel analogs of the cannabinoid partial agonists BAY 59-3074 and CB25 (see **Chapter 2**), a simple, catalyst-free procedure for the alkylation of phloroglucinol monoaryl ethers was developed.

In order to synthesize the desired third-generation compounds, Williamson Ether Synthesis conditions were utilized to transform the previously described phloroglucinol monoaryl ethers to the corresponding alkyl ethers.[1] This method was chosen due to its broad scope and simplicity. The reaction proceeds via S_N2 mechanism between an *in situ* generated phenoxide anion nucleuphile and a primary alkyl halide **(Scheme 4.2)**.[2] Previous reports have demonstrated the utility of cesium salts for phenoxide generation, and therefore cesium carbonate was chosen as the base for our procedure.[3] Additionally, consistent with most S_N2 mediated processes, the reaction has been proven to proceed well in polar aprotic solvents due to their ability to effectively solvate the free nucleuphile.[2] Given our previous success with N-methylpyrrolidone (NMP), the solvent was initially

chosen for this reaction as well, however, due to apparent solubility issues with certain substrates, acetonitrile was used instead with acceptable results.[1]

Scheme 4.2 General Williamson Ether Synthesis mechanism

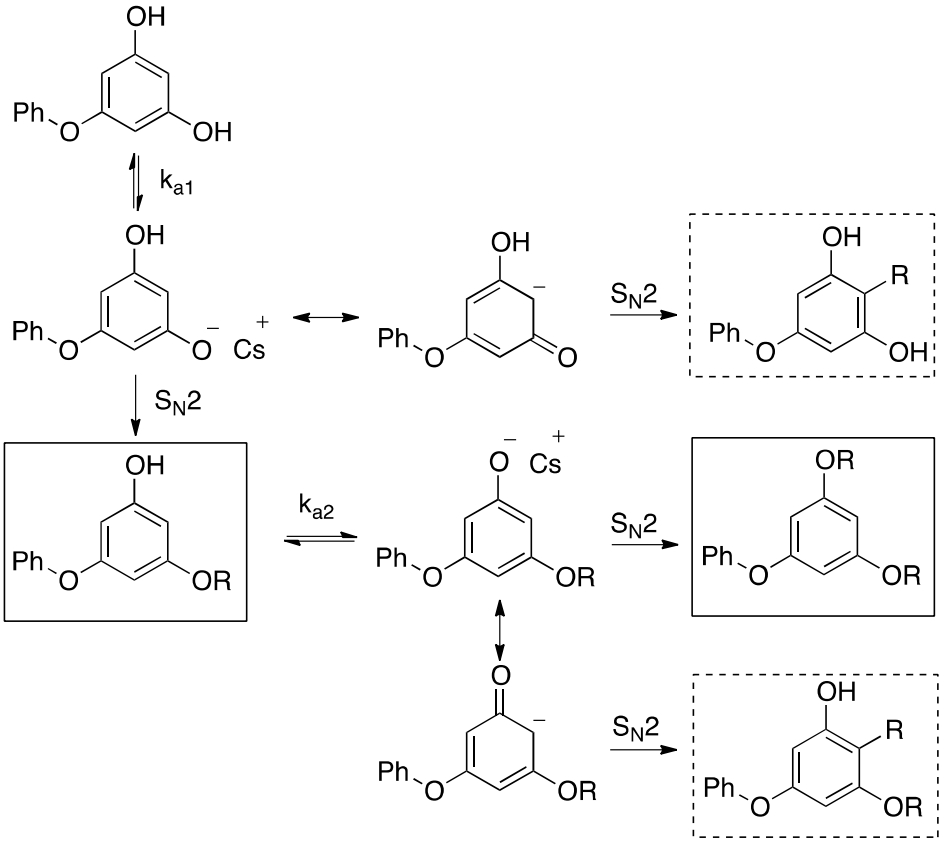


4.3 Results and Discussion

As summarized in Table 1, uncatalyzed William Ether Synthesis conditions afforded the desired mono alkyl ethers in moderate to low yield, which is typical for reactions of this nature.[4] Lower product yield was largely a result of the complex kinetics of the reaction, and are partially described in **Scheme 4.3**: The reactive nature of phloroglucinol mono aryl ether under alkaline conditions can be attributed in part to anionic delocalization of the phenoxide ion and therefore several competing side reactions were possible in the presence of alkyl halide; any intermediate product still bearing a phenolic hydroxyl group is capable of further deprotonation under basic conditions. The most facile side reaction was apparently formation of the di-alkyl ether; as demonstrated in **Tables 4.1** and **4.2**, recovery of the di-alkyl ethers either exceeded or was close to that of the mono-alkyl ethers. Furthermore, given that $K_{a2} > K_{a1}$, it is not surprising that a significant amount of di-alkyl ether would be produced. To a lesser extent C-alkylation was also theoretically possible, demonstrated by the resonance structures of generated phenoxide ions. Initially, out of interest in broadening the scope of the SAR studies, these di-alkyl ethers **4b-8b** were isolated and characterized in addition to the target mono-alkyl ethers. However, the highly

lipophilic di-alkyl ethers demonstrated little promise as cannabinoid receptor ligands and were not isolated and characterized in subsequent reactions. Though no C-alkylated products were formally characterized, the analysis of TLC and NMR spectra of crude reaction mixtures provided strong evidence of a mixture of C-alkylated products in trace amounts.

Scheme 4.3. Partial kinetics of the alkylation of phloroglucinol monoaryl ethers

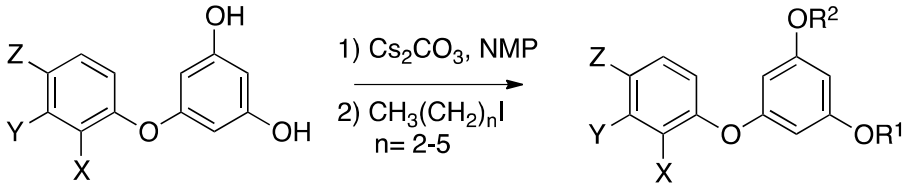


In an attempt to favor the formation of the desired mono-alkyl ether, the stoichiometry was manipulated in some examples such that there was an excess of phloroglucinol monoaryl ether. Statistically, this would lead to a higher probability of the first alkylation occurring. Though reaction conditions were not rigorously experimentally

optimized, it was found that approximately one-half equivalent of base and two equivalents of alkyl halide relative to phloroglucinol monoaryl ether produced acceptable mono-alkyl ether formation. Small changes to the ratios of starting materials were made across the reactions attempted, however, statistically significant changes in product yield were not observed. Conceivably, other variables such as reaction temperature, reaction time, phase of the moon and volatilization of alkyl halide also influenced product yield. In general, unreacted starting material could be recovered from all of the reactions attempted for recycling if desired. Given that the ultimate goal of this project was to make compounds available for biological screening, requiring relatively little material, further optimization was deemed unnecessary in the interest of time.

NMP was initially chosen as the solvent for these reactions given it's favorable properties as a polar aprotic solvent and based on our previous success using it for nucleophilic aromatic substitution conditions. The synthesis of products **4.1 - 4.4** was relatively facile in NMP. Unexpectedly, however, producing the desired mono and di-alkyl ethers **4.5 – 4.8** from the corresponding di-chloro substituted phloroglucinol mono aryl ether became increasingly difficult using the same reaction conditions. Curiously, product recovery systematically decreased with increasing carbon chain length of the alkyl halide used whereas this trend was not observed in the corresponding examples of products **4.1 - 4.4**. A reasonable explanation for this trend could not be imagined. It was later found that substituting acetonitrile for NMP as the solvent circumvented these somewhat confounding results.

Table 4.1 Alkylation of phloroglucinol mono aryl ethers in NMP

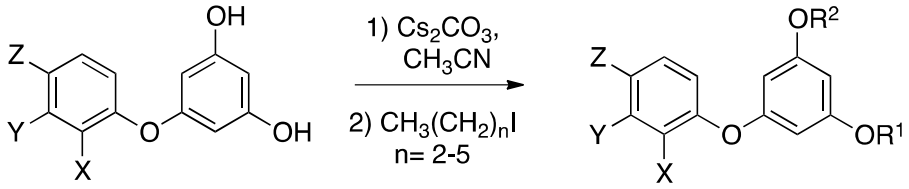


Cpd	Code	X	Y	Z	R^1	R^2	Yield (%)[a]
4.1a	AMS151	CN	CF_3	H	H	C_3H_7	18
4.1b	AMS150	CN	CF_3	H	C_3H_7	C_3H_7	47
4.2a	AMS159	CN	CF_3	H	H	C_4H_9	24
4.2b	AMS158	CN	CF_3	H	C_4H_9	C_4H_9	40
4.3a	AMS205	CN	CF_3	H	H	C_5H_{11}	17
4.3b	AMS204	CN	CF_3	H	C_5H_{11}	C_5H_{11}	ni[b]
4.4a	AMS167	CN	CF_3	H	H	C_6H_{13}	34
4.4b	AMS166	CN	CF_3	H	C_6H_{13}	C_6H_{13}	22
4.5a	AMS195	Cl	H	Cl	H	C_3H_7	15
4.5b	AMS194	Cl	H	Cl	C_3H_7	C_3H_7	ni[b]
4.6a	AMS197	Cl	H	Cl	H	C_4H_9	10
4.6b	AMS196	Cl	H	Cl	C_4H_9	C_4H_9	ni[b]
4.7a	AMS199	Cl	H	Cl	H	C_5H_{11}	14
4.7b	AMS198	Cl	H	Cl	C_5H_{11}	C_5H_{11}	ni[b]
4.8a	AMS215	Cl	H	Cl	H	C_6H_{13}	trace

[a] Isolated yield. [b]Product formation evident by TLC analysis, but not isolated or further characterized.

As summarized in **Table 4.2**, products synthesized in an acetonitrile solvent system were reliably afforded in 19% - 26% yield. Product **4.8a**, which could not be isolated using NMP **(Table 4.1)**, was produced in 24% yield using acetonitrile **(Table 4.2)**. While maximal yields were observed using NMP, the results were highly variable even under very similar reaction conditions. Conversely, the reactions carried out in acetonitrile produced slightly lower yields, but the experiments were very consistent and reliably reproducible. It is therefore recommended that any subsequent reactions be carried out in acetonitrile.

Table 4.2. Alkylation of phloroglucinol mono aryl ethers in acetonitrile



Cpd	Code	X	Y	Z	R^1	R^2	Yield (%)[a]
4.8a	AMS215	Cl	H	Cl	H	C_6H_{13}	24
4.9a	AMS2251	Cl	Cl	H	H	C_3H_7	23
4.10a	AMS2252	Cl	Cl	H	H	C_4H_9	22
4.11a	AMS2253	Cl	Cl	H	H	C_5H_{11}	20
4.12a	AMS2254	Cl	Cl	H	H	C_6H_{13}	27
4.13a	AMS2271	H	Cl	Cl	H	C_3H_7	22
4.14a	AMS2272	H	Cl	Cl	H	C_4H_9	19
4.15a	AMS2273	H	Cl	Cl	H	C_5H_{11}	22
4.16a	AMS2274	H	Cl	Cl	H	C_6H_{13}	23
4.17a	RS43	H	H	NO_2	H	C_4H_9	20
4.18a	RS39	H	H	NO_2	H	C_5H_{11}	18
4.19a	RS37	H	H	NO_2	H	C_6H_{13}	13

[a] Isolated yield.

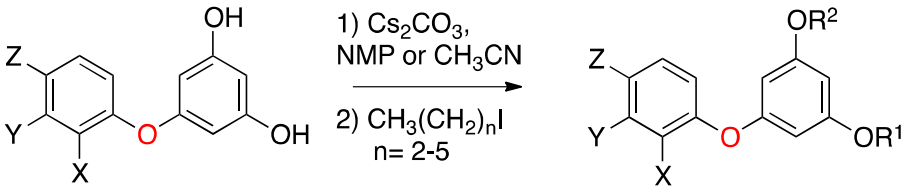
4.4 Conclusion

A simple method for the preparation of phloroglucinol monoaryl alkyl ethers from phloroglucinol monoaryl ethers has been developed. The compounds were synthesized with the ultimate goal of testing their activity at the cannabinoid CB1 receptor.

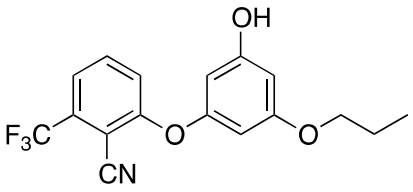
4.5 Acknowledgement

This research was funded by the National Institute on Drug Abuse (DA023916) and the University of New Orleans. Richard L. Schroeder assisted in the work presented here as part of his CHEM 3027 requirements.

4.6 Experimental Section

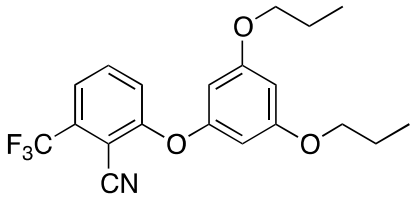


General Method A (Williamson-type alkylation) To phloroglucinol monoaryl ether (1.0 - 2.5 equiv.) dissolved in acetonitrile or N-methylpyrrolidinone (5 – 10 mL/mmol) was added Cs_2CO_3 (1.0 – 3.0 equiv). A condenser was placed onto the flask and the mixture was allowed to stir for 1 hour at 70 °C, which evolved CO_2 gas and generated a dark colored phenoxide solution. Finally, alkyl iodide or alkyl bromide (1.0 – 3.0 equiv.) was added through the condenser via syringe. The reaction was allowed to stir at 70°C overnight. The reaction mixture was cooled to room temperature then poured over H_2O (10 mL) resulting in a red colored suspension. HCl (6N) was added dropwise until the solution appeared slightly acidic on pH paper. The resulting aqueous mixture was extracted with Et_2O (3 x 15 mL). Pooled organic extracts were washed once with brine, dried over $MgSO_4$ and concentrated under reduced pressure on a rotoevaporator. The resulting residue was purified by preparative thin layer chromatography.



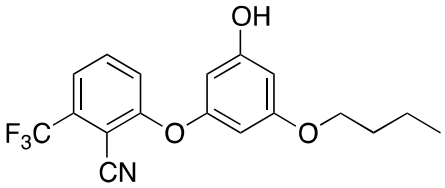
2-(3-hydroxy-5-propoxyphenoxy)-6-(trifluoromethyl)benzonitrile (4.1a)

General Method A. 2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (0.10 g, 0.34 mmol) was reacted with Cs_2CO_3 (0.19 g, 0.60 mmol) in NMP (1.5mL) for one hour then 1-iodopropane (0.10 g, 0.61 mmol) was added dropwise. Purification by preparative thin layer chromatography (20% ethyl acetate/hexane), R_f = 0.25, afforded **4.1a** (20 mg, 18%) as an off-white waxy solid, mp 65-66 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.54 (t, *J* = 8.1 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.31 (t, *J* = 2.2 Hz, 1H), 6.19 (t, *J* = 2.1 Hz, 1H), 6.14 (t, *J* = 2.1 Hz, 1H), 3.84 (t, *J* = 6.6 Hz, 2H), 1.76 (d, *J* = 7.3 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 161.78, 161.75, 159.6, 156.0, 135.6, 134.0, 122.8, 120.4, 120.10, 120.04, 112.6, 100.5, 99.8, 98.73, 98.69, 70.0, 22.7, 10.7. Anal. Calcd. for $C_{17}H_{14}F_3NO_3$: C, 60.54; H, 4.18; N, 4.15. Found: C; 60.76; H, 4.29; N, 3.95.



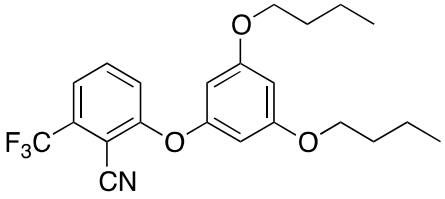
2-(3,5-dipropoxyphenoxy)-6-(trifluoromethyl)benzonitrile (4.1b)

General Method A. The production of **4.1b** was a competing reaction with **4.1a** and was therefore prepared concurrently. **4.1b** (60 mg, 47%) was isolated as the higher-R_f band on the preparative chromatography plate as a fluffy white solid, mp 61 - 61 °C. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.57 (t, *J* = 8.1 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.35 (s, 1H), 6.22 (d, *J* = 1.9 Hz, 2H), 3.87 (t, *J* = 6.5 Hz, 4H), 1.78 (dq, *J* = 14.0, 7.0 Hz, 4H), 1.01 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.7, 161.6, 156.1, 134.3, 134.1, 123.8, 121.0, 120.4, 120.2(q, 5Hz), 112.5, 101.0, 99.3, 98.9, 70.1, 22.1, 10.7. Anal. Calcd. for $C_{20}H_{20}F_3NO_3$: C, 63.32; H, 5.31; N, 3.69. Found: C; 63.03; H, 5.36; N, 3.59.



2-(3-butoxy-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (4.2a)

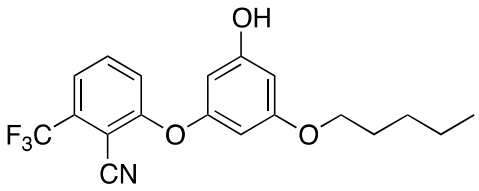
General Method A. 2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (0.25 g, 0.85 mmol) was reacted with Cs_2CO_3 (0.20 g, 0.61 mmol) in NMP (7.5 mL) for one hour then 1-iodobutane (0.10 g, 0.61 mmol) was added dropwise. Purification by preparative thin layer chromatography (30% ethyl acetate/hexane), R_f = 0.3, afforded **4.2a** (52 mg, 24 %) as a tan solid, mp 56-58 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.57 (t, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.30 (s, 1H), 6.19 (d, *J* = 1.0 Hz, 2H), 5.91 (s, 1H), 3.89 (t, *J* = 6.4 Hz, 2H), 1.73 (quintet, *J* = 7.1 Hz, 2H), 1.45 (dq, *J* = 14.9, 7.4 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 161.8, 161.6, 158.4, 156.1, 134.7, 134.3, 123.7, 121.0, 120.46, 120.34, 112.6, 100.3, 99.8, 99.5, 68.4, 31.3, 19.4, 14.0.



2-(3,5-dibutoxyphenoxy)-6-(trifluoromethyl)benzonitrile (4.2b)

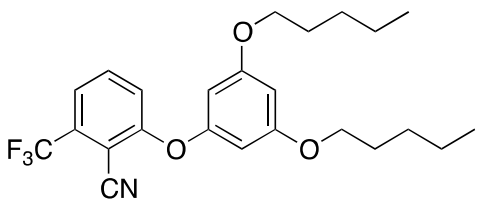
General Method A. The production of **4.2b** was a competing reaction with **4.2a** and was prepared concurrently. **4.2b** (100 mg, 40%) was isolated as the higher-R_f band on the preparative chromatography plate as a tan solid, mp 36 - 37 °C. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.57 (t, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.35 (s, 1H), 6.22 (s,

2H), 3.91 (t, *J* = 6.5 Hz, 4H), 1.74 (dt, *J* = 14.4, 7.0 Hz, 4H), 1.47 (dq, *J* = 15.0, 7.5 Hz, 4H), 0.96 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.7, 161.6, 156.1, 134.3, 134.1, 123.8, 121.0, 120.4, 120.2(q, 5Hz), 112.5, 101.0, 99.3, 98.9, 68.3, 31.4, 19.4, 14.0.



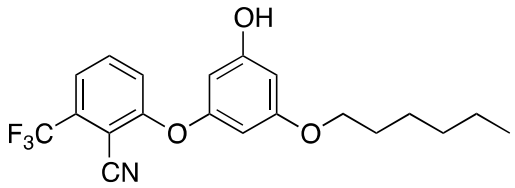
2-(3-hydroxy-5-(pentyloxy)phenoxy)-6-(trifluoromethyl)benzonitrile (4.3a)

General Method A. 2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (0.25 g, 0.85 mmol) was reacted with Cs_2CO_3 (0.10 g, 0.31 mmol) in NMP (6.0 mL) for one hour then 1-bromopentane (0.15 g, 1.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (30% ethyl acetate/hexane) afforded **4.3a** (54 mg, 17 %) as a white solid, mp 76-78 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.58 (t, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 6.29 (t, *J* = 2.1 Hz, 1H), 6.22 (t, *J* = 2.1 Hz, 1H), 6.18 (t, *J* = 2.1 Hz, 1H), 3.90 (t, *J* = 6.5 Hz, 2H), 1.76 (dt, *J* = 14.2, 7.0 Hz, 2H), 1.44-1.36 (m, 4H), 0.93 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 161.9, 161.4, 158.0, 156.3, 134.08, 134.05, 126.4, 120.53, 120.45, 120.40, 109.9, 100.2, 99.67, 99.57, 99.48, 68.7, 29.0, 28.3, 22.6, 14.2 Anal. Calcd. for $C_{19}H_{18}F_3NO_3$: C, 62.46; H, 4.97; N, 3.83. Found: C; 62.16; H, 5.02; N, 3.77.



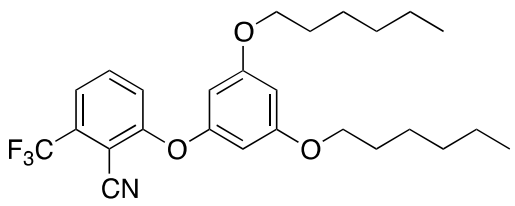
2-(3,5-bis(pentyloxy)phenoxy)-6-(trifluoromethyl)benzonitrile (4.3b)

General Method A. The production of **4.3b** was a competing reaction with **4.3a** and was prepared concurrently. Formation of **4.3b** was evident by TLC analysis R_f = 0.8 (25 % ethyl acetate/hexane), but not isolated or further characterized.



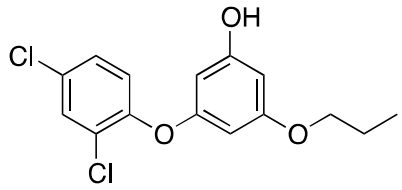
2-(3-(hexyloxy)-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (4.4a)

General Method A. 2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (0.25 g, 0.85 mmol) was reacted with Cs_2CO_3 (0.20 g, 0.61 mmol) in NMP (7.5 mL) for one hour then 1-iodohexane (0.13 g, 0.61 mmol) was added dropwise according to general procedure C. Purification by preparative thin layer chromatography (30% ethyl acetate/hexane) afforded **4.4a** (80 mg, 34 %) as a white waxy solid, mp 80-82 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.58 (t, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 6.30 (t, *J* = 2.0 Hz, 1H), 6.21 (t, *J* = 2.0 Hz, 1H), 6.19 (t, *J* = 2.0 Hz, 1H), 5.40 (s, 1H), 3.90 (t, *J* = 6.6 Hz, 2H), 1.75 (quintet, *J* = 7.3 Hz, 2H), 1.47-1.39 (m, 2H), 1.36-1.30 (m, 4H), 0.90 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.8, 161.6, 158.5, 156.1, 134.6, 134.3, 123.7, 121.0, 120.4, 120.3(q, 5Hz), 112.6, 100.3, 99.8, 99.4, 68.7, 31.7, 29.2, 25.8, 22.8, 14.2. Anal. Calcd. for $C_{20}H_{20}F_3NO_3$: C, 63.32; H, 5.31; N, 3.69. Found: C; 64.29; H, 5.85; N, 3.44.



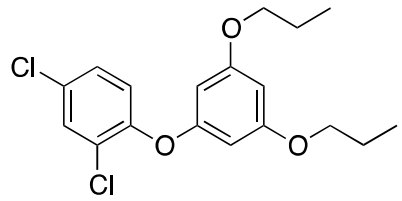
2-(3,5-bis(hexyloxy)phenoxy)-6-(trifluoromethyl)benzonitrile (4.4b)

General Method A. The production of **4.4b** was a competing reaction with **4.4a** and was therefore prepared concurrently. **4.4b** (62 mg, 22 %) was isolated as the higher-R_f band on the preparative chromatography plate a pink solid, mp 30 - 32 °C. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.57 (t, *J* = 8.2 Hz, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H), 6.34 (t, *J* = 2.0 Hz, 1H), 6.21 (d, *J* = 2.1 Hz, 2H), 3.89 (t, *J* = 6.5 Hz, 4H), 1.74 (dt, *J* = 14.5, 7.0 Hz, 4H), 1.46-1.39 (m, 4H), 1.31 (dt, *J* = 7.2, 3.6 Hz, 8H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 161.72, 161.61, 156.1, 134.5, 134.2, 123.8, 121.1, 120.45, 120.26(q, 5Hz), 112.5, 100.9, 99.2, 98.9, 68.5, 31.7, 29.3, 25.9, 22.8, 14.2.



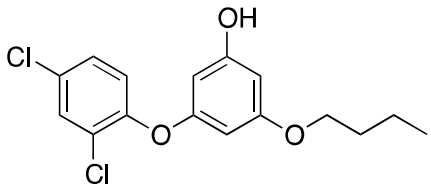
3-(2,4-dichlorophenoxy)-5-propoxyphenol (4.5a)

General Method A. 5-(2,4-dichlorophenoxy)benzene-1,3-diol (0.27 g, 1.0 mmol) was reacted with Cs_2CO_3 (0.33 g, 1.0 mmol) in NMP (7.5 mL) for one hour then 1-iodopropane (0.26g, 1.5 mmol) was added dropwise according to general procedure C. Purification by preparative thin layer chromatography (30% ethyl acetate/hexane) afforded **4.5a** (46 mg, 15%) as an amber oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.45 (d, *J* = 2.5 Hz, 1H), 7.19 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 6.16 (t, *J* = 2.1 Hz, 1H), 6.08 (t, *J* = 2.1 Hz, 1H), 6.00 (t, *J* = 2.1 Hz, 1H), 5.31 (s, 1H), 3.84 (t, *J* = 6.6 Hz, 2H), 1.76 (q, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H)..^{13}C NMR (100 MHz; $CDCl_3$): δ 161.6, 158.8, 157.8, 151.0, 130.6, 129.8, 128.3, 127.1, 122.4, 97.8, 97.6, 97.3, 70.0, 22.7, 10.7



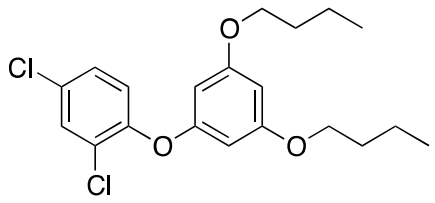
2,4-dichloro-1-(3,5-dipropoxyphenoxy)benzene (4.5b)

General Method A. The production of **4.5b** was a competing reaction with **4.5a** and was prepared concurrently. Formation of **4.5b** was evident by TLC analysis R_f = 0.8 (20 % ethyl acetate/hexane), but not isolated or further characterized.



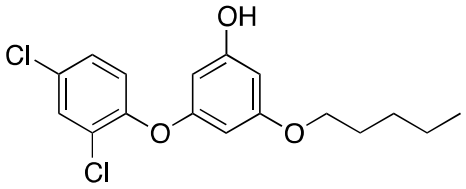
3-butoxy-5-(2,4-dichlorophenoxy)phenol (4.6a)

General Method A. 5-(2,4-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.35 g, 1.1 mmol) in NMP (7.5 mL) for one hour then 1-iodobutane (0.37g, 2.0 mmol) was added dropwise according to general procedure C. Purification by preparative thin layer chromatography (20% ethyl acetate/hexane) afforded **4.6a** (30 mg, 10 %) as a light yellow solid, mp 69-71 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.45 (d, *J* = 2.5 Hz, 1H), 7.20 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 6.15 (t, *J* = 2.2 Hz, 1H), 6.08 (t, *J* = 2.1 Hz, 1H), 6.00 (t, *J* = 2.2 Hz, 1H), 5.07 (s, 1H), 3.88 (t, *J* = 6.5 Hz, 2H), 1.72 (dt, *J* = 14.6, 7.0 Hz, 2H), 1.45 (dq, *J* = 15.0, 7.5 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 161.6, 158.9, 157.6, 151.0, 130.7, 129.8, 128.3, 127.2, 122.4, 97.7, 97.5, 97.3, 68.2, 31.4, 19.4, 14.0. Anal. Calcd. for $C_{16}H_{16}Cl_2O_3$: C, 58.73; H, 4.93. Found: C; 58.76; H, 5.03.



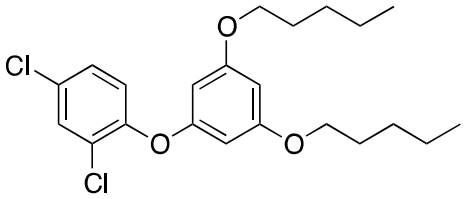
2,4-dichloro-1-(3,5-dibutoxyphenoxy)benzene (4.6b)

General Method A. The production of **4.6b** was a competing reaction with **4.6a** and was prepared concurrently. Formation of **4.6b** was evident by TLC analysis, R_f = 0.8 (20 % ethyl acetate/hexane), but not isolated or further characterized.



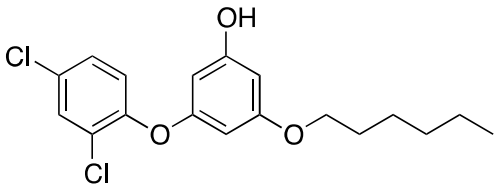
3-(2,4-dichlorophenoxy)-5-(pentyloxy)phenol (4.7a)

General Method A. 5-(2,4-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.35 g, 1.1 mmol) in NMP (7.5 mL) for one hour then 1-bromopentane (0.31 g, 2.0 mmol) was added dropwise according to general procedure C. Purification by preparative thin layer chromatography (30% ethyl acetate/hexane) afforded **4.7a** (43 mg, 14 %) as a light yellow solid, mp 56-60 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.44 (d, *J* = 2.5 Hz, 1H), 7.18 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.17-6.01 (m, 3H), 3.86 (t, *J* = 6.6 Hz, 2H), 1.73 (quintet, *J* = 7.0 Hz, 2H), 1.44-1.31 (m, 4H), 0.92 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.5, 158.8, 157.8, 151.0, 130.6, 129.7, 128.3, 127.1, 122.3, 97.9, 97.6, 97.3, 68.5, 29.0, 28.3, 22.6, 14.2.



1-(3,5-bis(pentyloxy)phenoxy)-2,4-dichlorobenzene (4.7b)

General Method A. The production of **4.7b** was a competing reaction with **4.7a** and was prepared concurrently. Formation of **4.7b** was evident by TLC analysis, R_f = 0.8 (20 % ethyl acetate/hexane), but not isolated or further characterized.

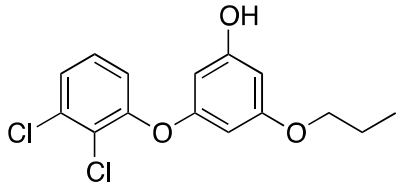


3-(2,4-dichlorophenoxy)-5-(hexyloxy)phenol (4.8a)

General Method A. 5-(2,4-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.20 g, 0.61 mmol) in NMP (7.5 mL) for one hour then 1-iodohexane (0.32 g, 1.5 mmol) was added dropwise. Purification by preparative thin layer chromatography (15% ethyl acetate/hexane) resulted in a trace amount of **4.8a.**

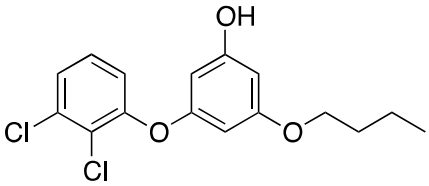
General Method A. 5-(2,4-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.20 g, 0.61 mmol) in acetonitrile (7.5 mL) for one hour then 1-iodohexane (0.32 g, 1.5 mmol) was added dropwise. Purification by preparative thin layer chromatography (15% ethyl acetate/hexane) afforded **4.8a** (80 mg, 24 %) as a light yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.44 (d, *J* = 2.4 Hz, 1H), 7.18 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 6.15 (s, 1H), 6.08 (s, 1H), 6.01 (s, 1H), 3.86 (t, *J* = 6.6 Hz, 2H), 1.74 (dd, *J* = 14.1, 7.2 Hz, 2H), 1.41 (dt, *J* = 14.2, 7.0 Hz, 2H), 1.31 (dt, *J* = 6.6, 3.5 Hz, 4H), 0.89 (t, *J* = 6.5

Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.6, 158.8, 157.7, 151.0, 130.7, 129.8, 128.3, 127.1, 122.3, 97.9, 97.6, 97.3, 68.5, 31.7, 29.3, 25.9, 22.8, 14.2.



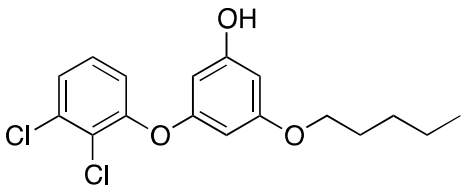
3-(2,3-dichlorophenoxy)-5-propoxyphenol (4.9a)

General Method A. 5-(2,3-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.15 g, 0.46 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodopropane (0.34 g, 2.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (15% ethyl acetate/hexane) afforded **4.9a** (65 mg, 23 %) as an amber oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.24 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 1H), 6.93 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.17 (t, *J* = 2.2 Hz, 1H), 6.10 (t, *J* = 2.1 Hz, 1H), 6.03 (t, *J* = 2.1 Hz, 1H), 5.77 (s, 1H), 3.83 (t, *J* = 6.6 Hz, 2H), 1.76 (sextet, *J* = 7.1 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.5, 158.6, 157.9, 153.8, 134.4, 127.8, 125.8, 125.4, 119.3, 98.2, 97.8, 97.5, 70.0, 22.9, 10.7. Anal. Calcd. for $C_{15}H_{14}Cl_2O_3$: C, 57.53; H, 4.51. Found: C; 57.51; H, 4.62.



3-butoxy-5-(2,3-dichlorophenoxy)phenol (4.10a)

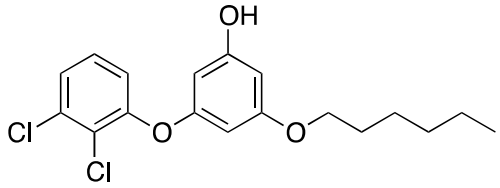
General Method A. 5-(2,3-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.15 g, 0.46 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodobutane (0.41 g, 2.2 mmol) was added dropwise. Purification by preparative thin layer chromatography (15% ethyl acetate/hexane) afforded **4.10a** (65 mg, 22 %) as an amber oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.24 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 1H), 6.93 (dd, *J* = 8.2, 1.4 Hz, 1H), 6.17 (t, *J* = 2.1 Hz, 1H), 6.10 (t, *J* = 2.1 Hz, 1H), 6.03 (t, *J* = 2.1 Hz, 1H), 5.83 (s, 1H), 3.87 (t, *J* = 6.5 Hz, 2H), 1.71 (dt, *J* = 14.5, 7.0 Hz, 2H), 1.45 (dt, *J* = 15.1, 7.5 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.5, 158.6, 157.7, 153.7, 134.4, 127.8, 125.9, 125.3, 119.3, 98.2, 97.8, 97.6, 68.3, 31.3, 19.4, 14.0. Anal. Calcd. for $C_{16}H_{16}Cl_2O_3$: C, 58.73; H, 4.93. Found: C; 58.90; H, 5.11.



3-(2,3-dichlorophenoxy)-5-(pentyloxy)phenol (4.11a)

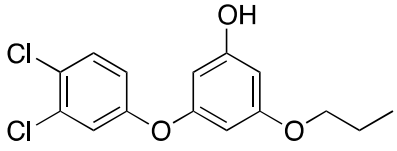
General Method A. 5-(2,3-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.15 g, 0.46 mmol) in acetonitrile (7.0 mL) for one hour then 1-bromopentane (0.28 g, 1.8 mmol) was added dropwise. Purification by preparative thin layer chromatography (15% ethyl acetate/hexane) afforded **4.11a** (62 mg, 20 %) as an amber oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.24 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 1H), 6.93 (dd, *J* = 8.2, 1.0 Hz, 1H), 6.17 (t, *J* = 2.0 Hz, 1H), 6.10 (t, *J* = 1.9 Hz, 1H), 6.03 (t, *J* = 1.9 Hz, 1H), 5.81 (s, 1H), 3.86 (t, *J* = 6.6 Hz, 2H), 1.73 (quintet, *J* = 7.0 Hz, 2H), 1.41-1.32 (m, 4H), 0.91 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.5, 158.6, 157.7, 153.7, 134.4,

127.8, 125.9, 125.3, 119.3, 98.2, 97.8, 97.6, 68.6, 29.0, 28.3, 22.6, 14.2. Anal. Calcd. for $C_{17}H_{18}Cl_2O_3$: C, 59.84; H, 5.32. Found: C; 59.96; H, 5.41.



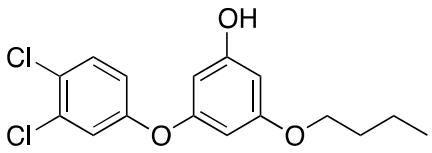
3-(2,3-dichlorophenoxy)-5-(hexyloxy)phenol (4.12a)

General Method A. 5-(2,3-dichlorophenoxy)benzene-1,3-diol (0.25 g, 0.92 mmol) was reacted with Cs_2CO_3 (0.15 g, 0.46 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodohexane (0.43 g, 2.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (15% ethyl acetate/hexane) afforded **4.12a** (87 mg, 27 %) as an amber oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.24 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 1H), 6.92 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.17 (t, *J* = 2.1 Hz, 1H), 6.10 (t, *J* = 2.1 Hz, 1H), 6.03 (t, *J* = 2.0 Hz, 1H), 5.95 (s, 1H), 3.86 (t, *J* = 6.6 Hz, 2H), 1.73 (dt, *J* = 14.5, 7.1 Hz, 2H), 1.41 (dt, *J* = 14.7, 7.2 Hz, 2H), 1.31 (dd, *J* = 7.1, 3.6 Hz, 4H), 0.89 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.5, 158.6, 157.7, 153.7, 134.4, 127.8, 125.9, 125.3, 119.3, 98.3, 97.8, 97.6, 68.6, 31.8, 29.3, 25.9, 22.8, 14.2. Anal. Calcd. for $C_{18}H_{20}Cl_2O_3$: C, 60.86; H, 5.67. Found: C; 60.91; H, 5.76.



3-(3,4-dichlorophenoxy)-5-propoxyphenol (4.13a)

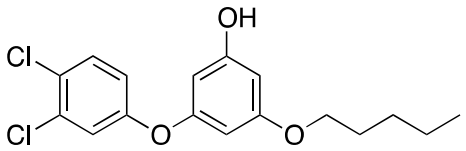
General Method A. 5-(3,4-dichlorophenoxy)benzene-1,3-diol (0.20 g, 0.74 mmol) was reacted with Cs_2CO_3 (0.12 g, 0.37 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodopropane (0.30 g, 1.8 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.13a** (51 mg, 22 %) as an amber oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.35 (d, *J* = 8.8 Hz, 1H), 7.10 (d, *J* = 2.8 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.19 (t, *J* = 2.1 Hz, 1H), 6.14 (t, *J* = 2.1 Hz, 1H), 6.08 (t, *J* = 2.1 Hz, 1H), 5.79 (s, 1H), 3.83 (t, *J* = 6.6 Hz, 2H), 1.76 (sextet, *J* = 7.1 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.6, 158.3, 157.8, 156.2, 133.4, 131.2, 127.0, 121.0, 118.6, 99.3, 98.6, 98.3, 70.1, 22.6, 10.7. Anal. Calcd. for $C_{15}H_{14}Cl_2O_3$: C, 57.53; H, 4.51. Found: C; 57.01; H, 4.64.



3-butoxy-5-(3,4-dichlorophenoxy)phenol (4.14a)

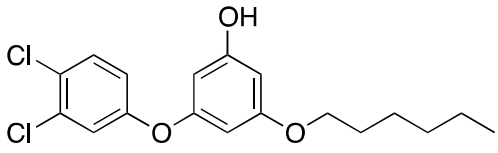
General Method A. 5-(3,4-dichlorophenoxy)benzene-1,3-diol (0.20 g, 0.74 mmol) was reacted with Cs_2CO_3 (0.12 g, 0.37 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodobutane (0.36 g, 2.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.14a** (46 mg, 19 %) as an amber oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.35 (d, J = 8.8 Hz, 1H), 7.10 (d, J = 2.8 Hz, 1H), 6.86 (dd, J = 8.8, 2.8 Hz, 1H), 6.20 (t, J = 2.1 Hz, 1H), 6.13 (t, J = 2.0 Hz, 1H), 6.08 (t, J = 2.1 Hz, 1H), 5.91 (s, 1H), 3.87 (t, J = 6.5 Hz, 2H), 1.72 (dt, J = 14.4, 7.0 Hz, 2H), 1.46 (dt, J = 15.1, 7.5 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H).^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.6, 158.3, 157.8, 156.2, 133.4, 131.2,

127.0, 121.0, 118.6, 99.3, 98.6, 98.3, 68.3, 31.3, 19.4, 14.0. Anal. Calcd. for $C_{16}H_{16}Cl_2O_3$: C, 58.73; H, 4.93. Found: C; 58.39; H, 5.02.



3-(3,4-dichlorophenoxy)-5-(pentyloxy)phenol (4.15a)

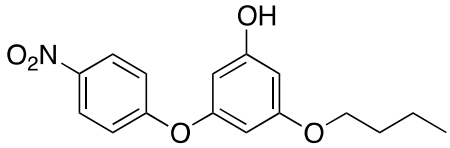
General Method A. 5-(3,4-dichlorophenoxy)benzene-1,3-diol (0.20 g, 0.74 mmol) was reacted with Cs_2CO_3 (0.12 g, 0.37 mmol) in acetonitrile (7.0 mL) for one hour then 1-bromopentane (0.25 g, 1.6 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.15a** (55 mg, 22 %) as a light yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.37 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 2.8 Hz, 1H), 6.87 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.19 (t, *J* = 2.2 Hz, 1H), 6.13 (t, *J* = 2.1 Hz, 1H), 6.07 (t, *J* = 2.1 Hz, 1H), 5.15 (s, 1H), 3.88 (t, *J* = 6.6 Hz, 2H), 1.75 (quintet, *J* = 7.1 Hz, 2H), 1.45-1.35 (m, 4H), 0.92 (t, *J* = 7.1 Hz, 3H).^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.7, 158.3, 157.8, 156.2, 133.4, 131.2, 127.0, 121.0, 118.7, 99.1, 98.6, 98.2, 68.5, 29.0, 28.3, 22.6, 14.2. Anal. Calcd. for $C_{17}H_{18}Cl_2O_3$: C, 59.84; H, 5.32. Found: C; 59.84; H, 5.40.



3-(3,4-dichlorophenoxy)-5-(hexyloxy)phenol (4.16a)

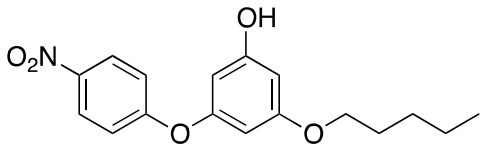
General Method A. 5-(3,4-dichlorophenoxy)benzene-1,3-diol (0.20 g, 0.74 mmol) was reacted with Cs_2CO_3 (0.12 g, 0.37 mmol) in acetonitrile (7.0 mL) for one hour then 1-

iodohexane (0.39 g, 1.8 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.16a** (60 mg, 23 %) as a light yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.35 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 2.8 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.19 (t, *J* = 2.2 Hz, 1H), 6.14 (t, *J* = 2.1 Hz, 1H), 6.07 (t, *J* = 2.1 Hz, 1H), 5.62 (s, 1H), 3.88 (d, *J* = 6.6 Hz, 2H), 1.74 (dt, *J* = 14.5, 7.1 Hz, 2H), 1.45-1.38 (m, 2H), 1.35-1.30 (m, 4H), 0.90 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.6, 158.3, 157.7, 156.2, 133.4, 131.2, 127.0, 121.0, 118.6, 99.1, 98.6, 68.6, 31.8, 29.3, 25.9, 22.8, 14.2. Anal. Calcd. for $C_{18}H_{20}Cl_2O_3$: C, 60.86; H, 5.67. Found: C; 60.80; H, 5.71.



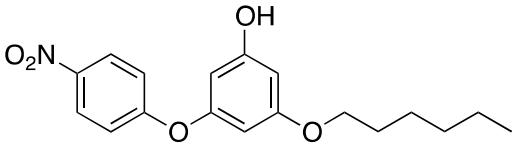
3-butoxy-5-(4-nitrophenoxy)phenol (4.17a)

General Method A. 5-(4-nitrophenoxy)benzene-1,3-diol (0.25 g, 1.0 mmol) was reacted with Cs_2CO_3 (0.17 g, 1.0 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodobutane (0.37 g, 2.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.17a** (60 mg, 20 %) as a yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.20 (d, *J* = 9.4 Hz, 2H), 7.05 (d, *J* = 9.4 Hz, 2H), 6.27 (s, 1H), 6.21 (s, 1H), 6.15 (s, 1H), 5.00 (s, 1H), 3.90 (t, *J* = 6.5 Hz, 2H), 1.74 (quintet, *J* = 7.2 Hz, 2H), 1.50-1.45 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 163.2, 161.9, 158.0, 156.7, 143.0, 126.1, 117.7, 100.4, 99.7, 99.3, 68.3, 31.3, 19.4, 14.0. Anal. Calcd. for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C; 62.70; H, 5.89; N, 4.42.



3-(4-nitrophenoxy)-5-(pentyloxy)phenol (4.18a)

General Method A. 5-(4-nitrophenoxy)benzene-1,3-diol (0.25 g, 1.0 mmol) was reacted with Cs_2CO_3 (0.17 g, 1.0 mmol) in acetonitrile (7.0 mL) for one hour then 1-bromopentane (0.30 g, 2.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.18a** (55 mg, 18 %) as a yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.20-8.16 (m, 2H), 7.06-7.02 (m, 2H), 6.28 (t, *J* = 2.1 Hz, 1H), 6.20 (t, *J* = 2.1 Hz, 1H), 6.16 (t, *J* = 2.1 Hz, 1H), 3.89 (t, *J* = 6.6 Hz, 2H), 1.79-1.72 (m, 2H), 1.44-1.38 (m, 4H), 0.91 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 163.2, 161.8, 158.1, 156.7, 142.9, 126.1, 117.7, 100.4, 99.7, 99.3, 68.6, 29.0, 28.3, 22.6, 14.2.



3-(hexyloxy)-5-(4-nitrophenoxy)phenol (4.19a) 5-(4-nitrophenoxy)benzene-1,3-diol (0.25 g, 1.0 mmol) was reacted with Cs_2CO_3 (0.17 g, 1.0 mmol) in acetonitrile (7.0 mL) for one hour then 1-iodobutane (0.42 g, 2.0 mmol) was added dropwise. Purification by preparative thin layer chromatography (25% ethyl acetate/hexane) afforded **4.19a** (42 mg, 13 %) as a light yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.21-8.17 (m, 2H), 7.06-7.02 (m, 2H), 6.28 (t, *J* = 2.2 Hz, 1H), 6.20 (t, *J* = 2.1 Hz, 1H), 6.16 (t, *J* = 2.1 Hz, 1H), 3.89 (t, *J* = 6.6 Hz, 2H), 1.75 (dt, *J* = 14.5, 7.1 Hz, 2H), 1.46-1.41 (m, 2H), 1.35-1.32 (m, 4H), 0.89 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 163.2, 161.8, 158.1, 156.7, 142.9, 126.1, 117.7, 100.4,

99.7, 99.3, 68.6, 31.7, 29.2, 25.9, 22.8, 14.2. Anal. Calcd. for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C; 64.47; H, 6.40; N, 4.07.

4.7 References

(1) Sherwood, A.; Pond, D.; Trudell, M. *Synthesis (Stuttg).* **2012**, *44*, 1208–1212.

(2) Boyd, Robert W.; Morrison, R. *Organic chemistry*; Prentice Hall: Englewood Cliffs, N.J, 1992; pp. 241–242.

(3) Lee, J. C.; Yuk, J. Y.; Cho, S. H. *Synth. Commun.* **1995**, *25*, 1367–1370.

(4) Brizzi, A.; Brizzi, V.; Cascio, M. G.; Corelli, F.; Guida, F.; Ligresti, A.; Maione, S.; Martinelli, A.; Pasquini, S.; Tuccinardi, T.; Di Marzo, V. *J. Med. Chem.* **2009**, *52*, 2506–2514.

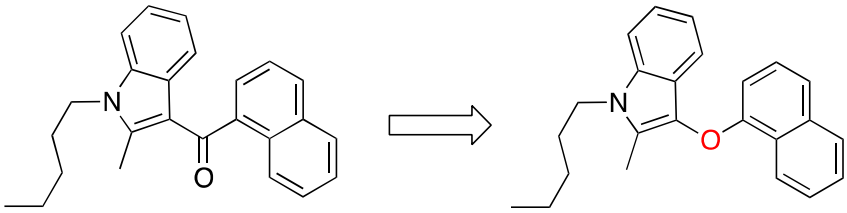
5

Synthesis of Indole aryl ethers as Cannabinoid Receptor Ligands

5.1 Abstract

A synthetic method has been developed to prepare the novel indole aryl ether analog of a napthoyl indole with known cannabinoid receptor activity. This compound was synthesized in order to test the hypothesis that the ether analog would also exhibit binding affinity at the CB1 receptor. The compound was synthesized in 4-steps with an overall unoptomized yield of 10%. Bioassay of the new compound demonstrated that it exhibited nanomolar binding affinity at the CB1 receptor.

Scheme 5.1. Abstract scheme: proposed structure for novel indole naphthol ether cannabinoids



prototypical napthoyl indole developed by Huffman
5.1

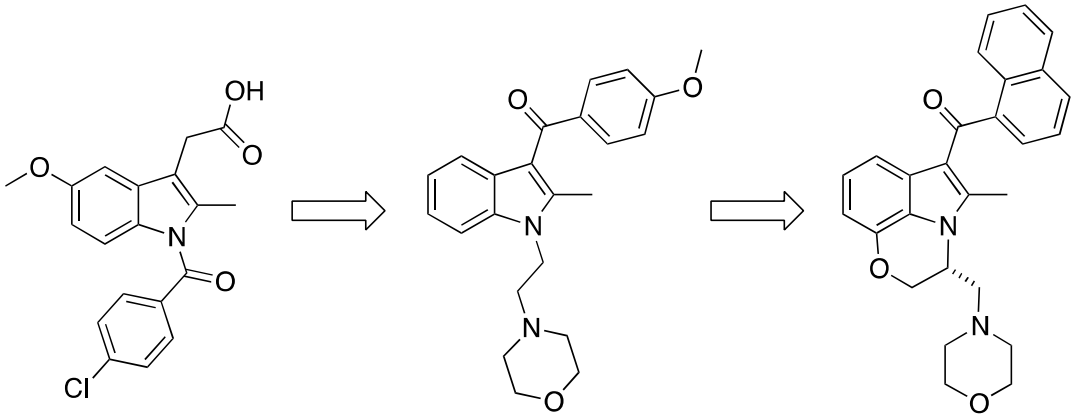
proposed novel indole aryl ether
5.2

5.2 Introduction

The non-classical napthoyl indole cannabinoids came about as a result of an interesting serendipitous discovery in the 1980's. A series of unexpected connections led to an entirely new, structurally distinct class of cannabinoid receptor ligands **(Scheme 5.2)**. The drug Indometacin **(5.3)** was discovered in 1963. It became (and still is) a prescribed non-steroidal anti-inflammatory drug (NSAID) that works by reducing prostaglandin production by nonselective inhibition of the enzymes cyclooxygenase 1 and 2. In the 1980's, researchers at the pharmaceutical company Sterling-Winthrop were searching for new analgesic NSAIDS and had synthesized some derivatives of **5.3**.[1] One of those compounds was Pravadoline **(5.4),** which worked similarly by inhibiting COX and so it demonstrated anti-inflammatory and analgesic properties. The analgesic properties of **5.4** were, however, unexpectedly high—at doses more than 10 times lower than the effective anti-inflammatory dose the compound displayed strong analgesic activity in rats, and therefore, a COX-mediated mechanism was unlikely.[2] In a logical step, the researchers administered **5.4** in combination with naloxone, an opioid receptor antagonist, to determine if the compound was reducing pain sensitivity in rats by interacting with opioid receptors, but the analgesic property remained so opioid activity was also unlikely. The researchers were unconvinced and tested the compound *in vitro* using radioligand displacement at opioid receptors but it did not bind at up to 10 μM concentrations. Furthermore, subsequent extensive testing in isolated tissue preparations and in radioligand binding assays had ruled out the interaction of **5.4** with muscarinic, cholinergic, adrenergic, serotonin, opioid, purinergic, dopaminergic, histaminergic,

glutaminergic, VIP, NPY, somatostatin, bombesin, GABAergic, neurokinin, bradykinin, and prostaglandin receptors.[3,4]

Scheme 5.2. From prescription NSAIDs to the first aminoalkyl indole cannabinoids



Indometacin (NSAID) **5.3**

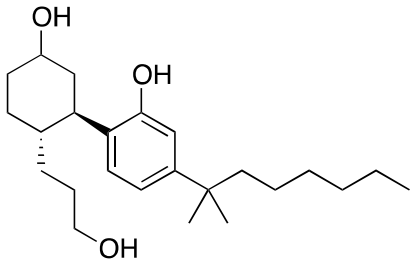
Pravadoline (NSAID + cannabinoid) **5.4**

WIN 55,212-2 (cannabinoid) **5.5**

In order to further explore this curious non-opioid antinociceptive NSAID, the researchers at Sterling-Winthrop conducted a structure activity relationship study of Pravadoline analogs resulting in several compounds that were devoid of the ability to inhibit prostaglandin synthesis (non NSAID) but still clearly demonstrated antinociceptive ability. For the first time, it was hypothesized (in passing) that the antinociceptive mechanism *might* be associated with binding to the recently characterized cannabinoid receptor.[2] A publication from the same group several months later produced another SAR study with the goal of supporting the hypothesis that the Pravadoline-derived compounds were cannabinoid receptor ligands by identifying molecular features that inhibit neuronally stimulated contractions in mouse vas deferens (MVD), an assay that responds well to cannabinoids, while minimizing the NSAID activity.[4] The most potent compound at

inhibiting MVD contractions to emerge from this study was WIN 55,212-2 **(5.5)**. Finally, ^{3}H-labeled **5.5** was used to demonstrate that the binding site distribution in the brain was functionally equivalent with the THC **(5.7)** derivative, CP-55940 (**5.6)** cannabinoid binding site.[4] And so, a new class of cannabinoid receptor ligands was born. The aminoalkylindole **5.5** is still a ubiquitous and useful tool in cannabinoid research today.

Figure 5.1. Synthetic THC derived cannabinoid, CP-55940

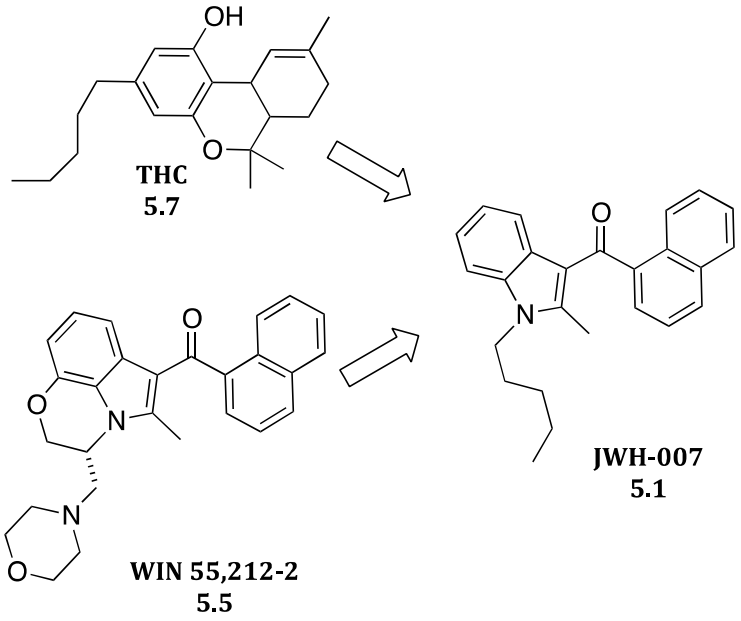


5.6

In the year 1994 following the introduction of WIN 55,212-2, Huffman *et al.* published an SAR demonstrating that compounds built around a napthoyl indole core bind to the same receptor as THC ("CB1" receptor was a relatively new term and doesn't actually appear in the paper, though it is the receptor that he was referring to).[5] Prompted by the fact that WIN 55,212 bared little apparent structural resemblance to THC or CP-55940, Huffman sought to identify molecular features required for binding affinity at the cannabinoid receptor by analyzing and comparing both THC and WIN 55,212-2 **(Scheme 5.3)**. Using computer aided molecular modeling, it was shown that the two compounds could be aligned and superimposed. Doing so placed the 5-carbon alkyl chain of THC near the nitrogen atom of the indole on **5.5**. It was therefore hypothesized that napthoyl indoles

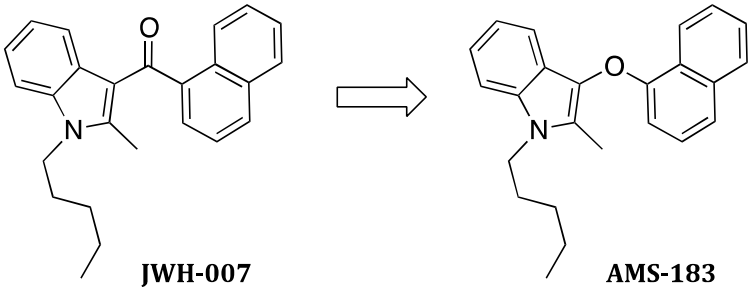
bearing hydrophobic alkyl chains at the indole nitrogen would show cannabinoid activity. The napthoyl indole **5.1** was the most potent of the series with a K_i value of 9.5 nM.

Scheme 5.3. Huffman's rationale for designing the aminoalkyl napthoyl indoles



The goals of the proceeding work were to develop a synthetic procedure able to produce a compound that would expand the SAR study conducted by Huffman as well as our own model of diaryl ether cannabinoids. Noting that **5.1** is essentially a diaryl ketone, we envisaged that the carbonyl linkage could simply be substituted by an ether linkage yielding alkylindole aryl ether **5.2** reminiscent of our previously described diaryl ethers.

Scheme 5.4. Indole aryl ether design strategy

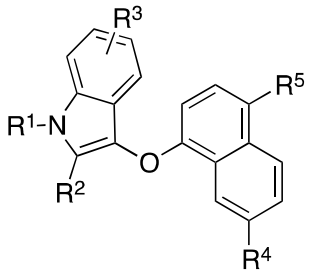


JWH-007
5.1

AMS-183
5.2

Though our synthetic procedure was used only to produce one representative compound, we hypothesize that upon substitution of the appropriate starting reagents, similar methodology could be used to produce a variety of compounds of the same form as **5.8**, thereby significantly expanding the structure activity relationship studies.

Figure 5.2. Indole aryl ether general scaffold



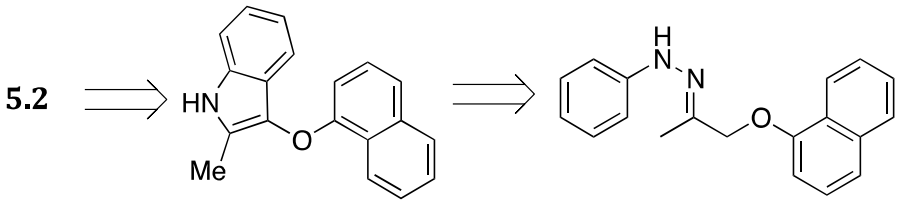
5.8

5.3 Results and Discussion

As illustrated by the retrosynthesis in **Scheme 5.5**, we envisioned that indole **5.9** would be the key precursor to our target, **5.2**, and could be produced by applying Fischer Indole Synthesis methodology to phenylhydrazone **5.10**. From key precursor **5.9**, S_N2-

mediated alkylation at the relatively basic amine was predicted to be facile, as it was the same approach used by Huffman to produce **5.1**.[6]

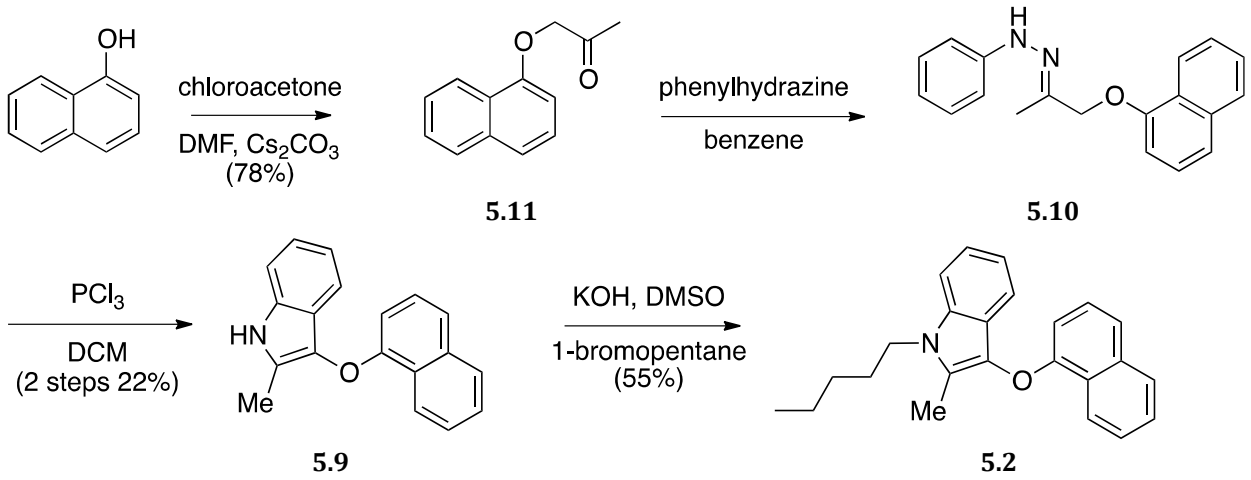
Scheme 5.5. Retrosynthesis to target compound 5.2



5.9 **5.10**

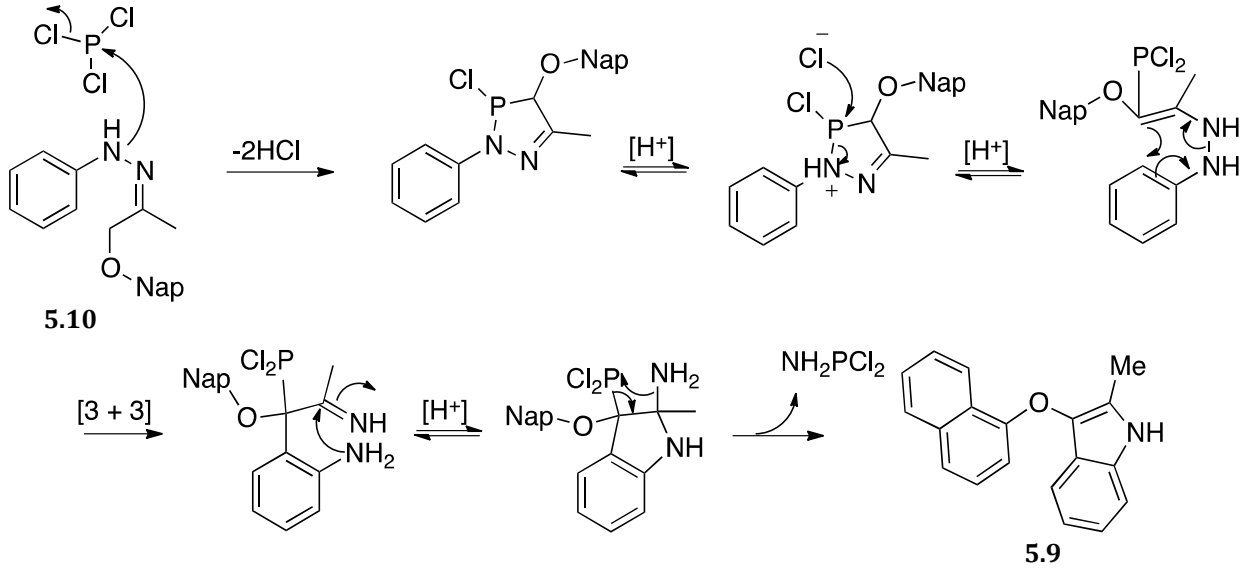
With the retrosynthesis described above in hand, the synthesis of target compound **5.2** was mostly straightforward (**Scheme 5.6**). The S_N2 product of 2-naphthol and chloroacetone afforded ketone **5.11** in high yield and acceptable purity directly from the workup. Phenylhydrozone **5.10** was readily afforded by the condensation of phenyl hydrazine with ketone **5.11** in benzene and was used subsequently without further purification. The indole synthesis reaction to **5.9** proceeded with moderate difficulty. The workup was somewhat tedious (refer to Experimental Section for further details) and the resulting crude residue required careful column chromatography in order to isolate **5.9** in reasonable purity, though conceivably the reaction could be optimized for better results. Alkylation of **5.9** was analogous to procedures described by Huffman previously and preceded without difficulty.[6]

Scheme 5.6. Synthetic route to target compound 5.2



It is also worth noting that very few literature examples exist describing the synthesis of indoles bearing ether linkages at the 3-position, and thus **5.9** represents a relatively unique novel scaffold that probably deserves some further attention in the future. The reaction presented here was based on an example found in patent literature that provided the synthesis of an indole somewhat related to **5.9**.[7] In the example presented, the reaction was carried out a room temperature and mediated by stoichiometric amounts of phosphorus trichloride, which are atypical conditions for a classical Fischer Indole synthesis, and likely proceeds via a unique mechanism that differs in several ways. As proposed by Todesco *et al.,* the reaction is likely mediated through a diazaphophole intermediate, ultimately ending with a Wittig-like elimination of NH_2PCl_2 to produce the indole.[8] For product **5.9**, the proposed mechanism is illustrated in **Scheme 5.7**.

Scheme 5.7. Proposed indole synthesis mechanism to key precursor 5.9



Radioligand displacement studies of **5.2** at CB1 receptors demonstrated a K_i value of 245 nM, suggesting that the compound has moderate to high binding affinity. A full analysis and discussion of the bioassay data from compound **5.2** is presented in **Chapter 6** within the context of the entire diaryl ether SAR studies.

5.4 Conclusion

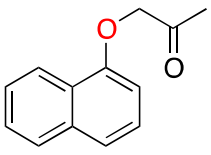
We have demonstrated for the first time that N-alkyl indole aryl ethers may represent a new class of potent cannabinoid receptor ligands. Though our novel synthetic strategy was used only to produce one representative compound, we hypothesize that upon substitution of the appropriate starting reagents, similar methodology could be used to produce a variety of compounds resulting in an interesting cannabinoid SAR study.

5.5 Acknowledgement

This research was funded by the National Institute on Drug Abuse (DA023916) and the University of New Orleans.

5.6 Experimental Section

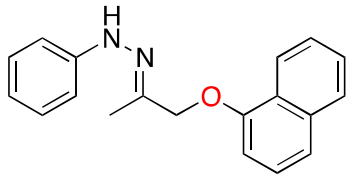
All chemicals were purchased from Aldrich Chemical Company and used as received unless otherwise noted. TLC: silica gel (250 μm); visualization with UV light, I_2, or phosphomolybdic acid. Chromatography: silica gel 60 Å (230–400 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian 400 MHz NMR spectrometer at ambient temperature in $CDCl_3$ or DMSO-d_6. ^{1}H NMR chemical shifts are reported as δ values (ppm) relative to TMS. ^{13}C NMR chemical shifts are reported as δ values (ppm) relative to $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.5 ppm). Melting points were recorded on a Mel-temp apparatus and are uncorrected. Atlantic Microlab, Inc., Norcross, GA performed all CHN microanalyses.



1-(naphthalen-1-yloxy)propan-2-one (5.11)

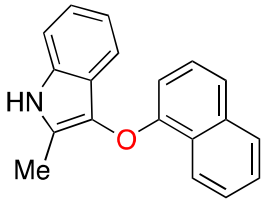
To a solution of 1-napthol (7.2 g, 0.05 mol) in DMF (40 mL) was added Cs_2CO_3 (16 g, 0.05 mol) at room temperature and a nitrogen atmosphere was established. The initial pink solution became blue/green and opaque after 15 minutes of stirring. Chloroacetone (4 mL,

0.05mol) was added via syringe slowly, resulting in an exothermic reaction. The reaction stirred at room temperature for 5h, over which time it became a dirty brown-green color. The resulting suspension was dumped into H_2O (75 mL) and extracted with Et_2O (2 x 75 mL). Combined ether extracts were washed once with NaOH (1N, 50 mL), brine (100 mL) and dried over Na_2SO_4. The extract was filtered and concentrated to afford **5.11** (7.9 g, 78%) as a thick amber oil that slowly formed flower shaped crystals upon sitting overnight. The compound was used subsequently without further purification. ^1H NMR ($CDCl_3$; 400 MHz) δ: 8.35 - 8.37 (m, 1H), 7.82 - 7.85 (m, 1H), 7.38 – 7.55 (m, 3H), 7.36 (t, J = 8.0 Hz, 1H), 6.66 – 6.67 (m, 1H), 4.67(s, 2H), 2.38 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 206.3, 153.7, 134.9, 127.8, 127.0, 125.9, 122.1, 121.6, 105.1, 73.4, 27.1.



1-(1-(naphthalen-1-yloxy)propan-2-ylidene)-2-phenylhydrazine (5.10)

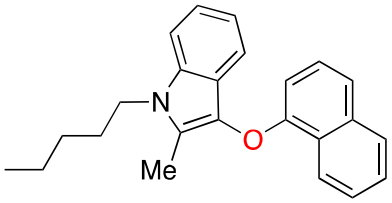
5.11 (2.0 g, 10 mmol) and phenylhydrazine (1.0 g, 9.0 mmol) were dissolved in benzene (10 mL). The clear orange solution became cloudy. The reaction was carried out under nitrogen for 1h. The reaction mixture was dried over Na_2SO_4, filtered and concentrated under reduced pressure on the rotary evaporator followed by high-vacuum overnight resulting in approximately 3 g of viscous red oil. The compound was used without further purification or characterization.



2-methyl-3-(naphthalen-1-yloxy)-1*H*-indole (5.09)

Compound **5.10** (3.0 g, 10 mmol) was dissolved in dry DCM (25 mL). PCl_3 (1.8 mL, 21 mmol) was syringed into the reaction and the mixture was allowed to stir at room temperature under nitrogen for 24h. The reaction was quenched by slowly introducing H_2O (~0.5 mL) and resulted in the formation of copious amounts of gaseous HCl. The reaction was allowed to stir for about 30 minutes until gas evolution ceased. The reaction mixture was brown with some black solids. NaOH (4N) was added until the pH of the solution appeared approximately neutral on pH paper. The entire mixture was concentrated under reduced pressure on a rotoevaporator. The resulting aqueous brown slurry was added to water (100 mL) and extraction was attempted with Et_2O. Suspended solids greatly impeded the extraction.* The aqueous mixture was extracted with four more volumes of Et_2O (100 mL). Combined organic extracts were washed with water followed by brine then dried over $MgSO_4$. Filtration and concentration resulted in 1.07 g of reddish brown oil. The oil was purified by column chromatography (10-20% EtOAc in Hexanes). The fractions corresponding to TLC: Rf = 0.6 in 20% EtOAc/Hexanes proved to be **5.09** (0.6 g, 22%) as a red oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.60-8.57 (m, 1H), 7.87 (dd, *J* = 6.7, 2.8 Hz, 1H), 7.69 (br s, 1H), 7.60-7.54 (m, 2H), 7.51-7.46 (m, 2H), 7.33-7.31 (m, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 7.15 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H), 7.01 (ddd, *J* = 7.9, 7.1, 0.9 Hz, 1H), 6.67 (dd, *J* = 7.7, 0.8 Hz, 1H), 2.36 (s, 3H).

*It is recommended that the workup/extraction outlined above be modified should this experiment be repeated. Quenching on ice would probably be wise. Filtration of the initial reaction mixture would probably result in a cleaner workup and an extraction solvent other than Et_2O might be better.



2-methyl-3-(naphthalen-1-yloxy)-1-pentyl-1*H*-indole (5.2)

5.09 (0.6 g, 2.2 mmol) was added to a stirred suspension of freshly powdered KOH (0.65 g, 11 mmol) in DMSO (2.5 mL). 1-bromopentane (0.56 mL, 4.4 mmol) was added and the reaction stirred at 80°C for 24h. The reaction mixture was poured into H_2O (10 mL) and extracted with ethyl acetate (3 x 10mL). Combined organic extracts were washed with water, brine, then dried over Na_2SO_4. Concentration of the extract resulted in 0.6 g of red oil. Flash column chromatography on silica (10:1 pet. ether/ EtOAc) yielded slightly impure **5.2** (0.4 g, 55 %) as a red oil.* In order to further purify the product, 200mg of this oil was subjected to preparative TLC (7% EtOAc/ Hexanes). A yellow-colored band at approximately R_f = 0.5 was isolated affording **5.2** (180 mg, 90%) as a yellow oil that spontaneously crystallized to a tan solid. ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.61-8.58 (m, 1H), 7.90-7.86 (m, 1H), 7.61-7.54 (m, 2H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.35 (dt, *J* = 8.3, 0.8 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.17 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.00 (ddd, *J* = 7.9, 7.0, 0.9 Hz, 1H), 6.64 (dd, *J* = 7.7, 0.9 Hz, 1H), 4.12 (t, *J* = 7.4 Hz, 2H), 1.81 (dt, *J* = 14.7,

7.4 Hz, 2H), 1.42-1.37 (m, 4H), 0.93 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 134.9, 133.93, 133.92, 127.8, 126.7, 126.2, 125.7, 122.5, 121.3, 121.2, 119.3, 117.6, 109.5, 107.6, 81.4, 75.8, 43.7, 30.3, 29.5, 22.8, 22.8, 14.3, 9.2.

Anal. Calcd. for $C_{24}H_{25}NO$: C, 83.93; H, 7.34; N, 4.08. Found: C; 83.34; H, 7.35; N, 4.02.

*Flash column chromatography in 100% petroleum ether would probably work to isolate pure **5.2** in one run rendering additional purification unnecessary.

5.7 References

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6

Bioassay and Structure Activity Relationship Studies of Diaryl Ethers at the CB_1 Receptor and the Discovery of a Novel High Affinity Low Efficacy Compound

6.1 Abstract

Compounds consisting of a diaryl ether core described in the previous four chapters were evaluated for activity at the cannabinoid CB1 receptor and binding affinity data for 34 new compounds is reported. The compounds were initially screened by radioligand displacement at two concentrations. Several moderate affinity ($K_i \sim 1$ μM) compounds emerged from initial studies and helped identify some functionality necessary for binding which helped drive the research forward. Subsequent bioassay led to the discovery of one very high affinity compound, AMS167, with a K_i value of 1.2 nM. This compound was subject to further testing where it was revealed that it behaved as a low efficacy compound. In support of a neutral antagonist / inverse agonist efficacy profile, AMS167 was found to block the behavioral effects of cannabinoid agonists in rats. With the available data, a partial structure activity relationship was discussed for diaryl ether cannabinoids.

6.2 Introduction

Low efficacy ligands that bind to the cannabinoid receptor represent an attractive target for various pharmacotherapies to treat CNS-related disorders including obesity and

addiction.[1,2] Furthermore, there are very few high affinity CB1 selective ligands that exhibit low efficacy profiles, and therefore, less is known about the CB1 partial agonist pharmacophore.

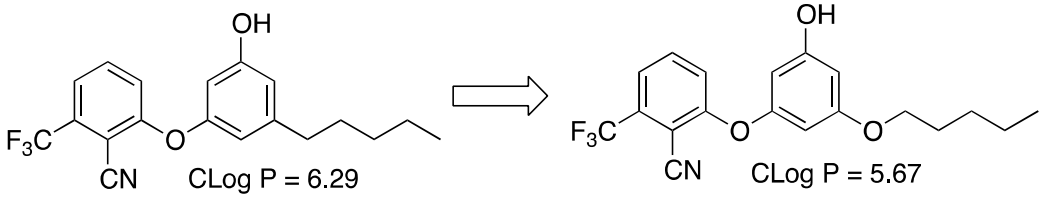
This report describes the structure activity relationships of side-chain modified diaryl ether hybrid derivatives of BAY 59-3074 (**2.2**) and CB25 (**2.3**) as summarized in **Chapter 2**. These hybrid derivative compounds were studied to determine structural features necessary for better binding affinity and improved solubility while retaining a partial agonist efficacy profile relative to their parent compounds.

Compounds exhibiting reasonable inhibition at two concentrations were advanced to full binding affinity studies and the corresponding K_i values were reported. Several moderate affinity (K_i < 1 μM) and one high affinity compound (K_i = 1.2 nM) emerged. The high affinity compound, AMS167, was advanced to efficacy studies and found to behave as a inverse agonist/neutral antagonist regarding locomotor activity in rats.

Scheme 6.1 outlines the ligand design rationale that was guided by the bioassay data presented here. In first-generation diaryl ether cannabinoids described in **Chapter 2**, it was evident that alkyl group R played a role in favorable interaction with the CB1 receptor. Also, the relatively low activity of second generation esters suggested the possibility that a phenolic hydroxyl group may be important for favorable binding, a hypothesis that is supported by the structural features of known cannabinoids such as THC (**1.16**) and CP-55,940 (**1.17**).[3] Third generation phloroglucinol-derived compounds were designed in interest of ideally decreasing lipophilicity by converting the alkyl side chains to alkyl ethers while simultaneously retaining the phenolic hydroxyl group with the ultimate goal of increasing binding affinity compared to their first generation analogs. Given that

compounds with high log P values are generally associated with poor blood-brain barrier penetrability, the more hydrophilic compounds would likely make better drug candidates.[4] Comparing representative second and third generation compounds, approximately 10 % reduction in calculated Log P was accomplished (**Figure 6.1**). In order to further expand our SAR, compounds bearing a variety of different substitutions (X, Y, Z) on the electron deficient aryl ring were also synthesized (**Chapters 3-4**).

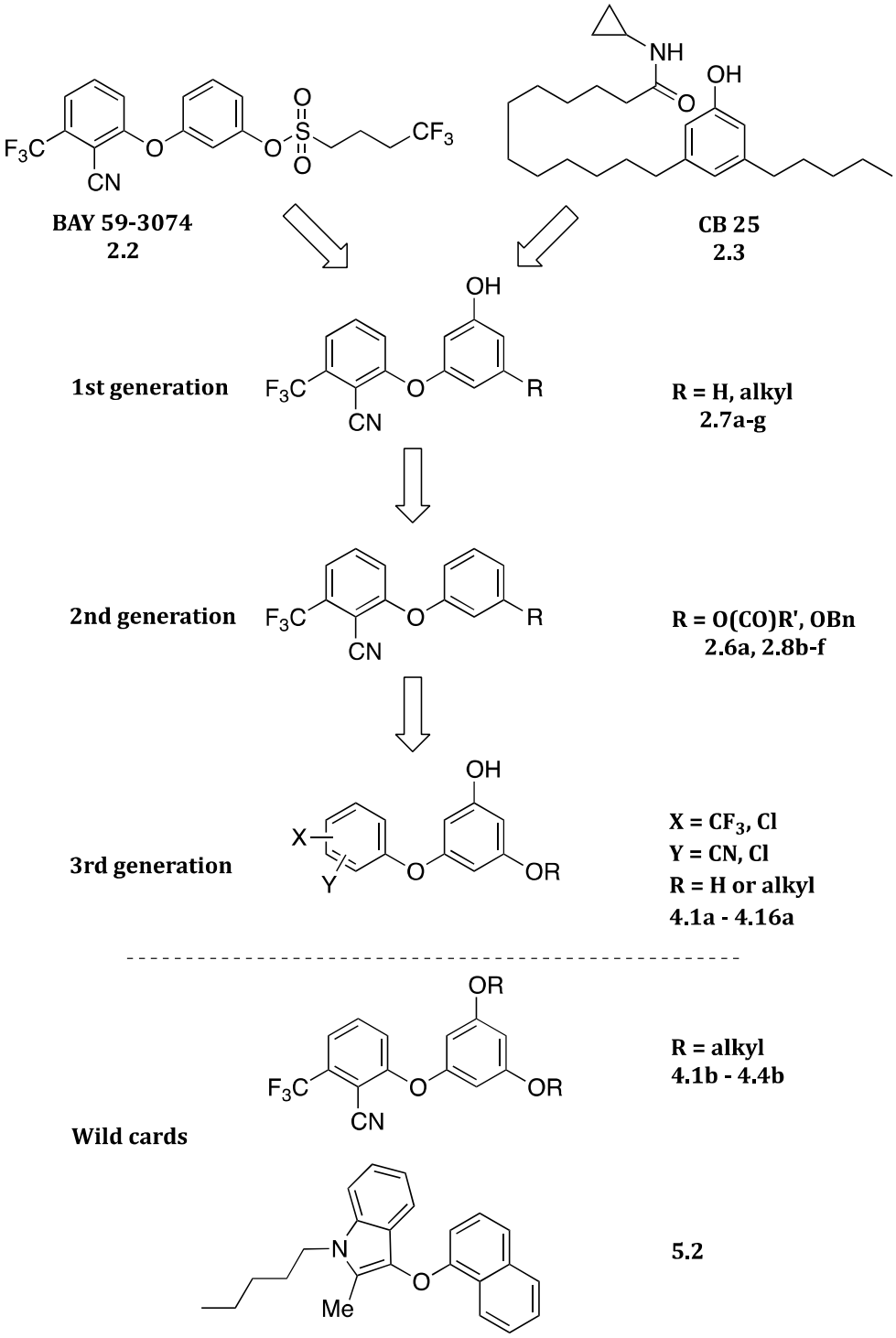
Figure 6.1. Calculated Log P constants for representative first and third generation compounds



2.7f **4.3a**

In addition to our rational design approach, several compounds were tangentially synthesized throughout the course of the project and were also evaluated for activity at CB1 receptors. Most of the so-called "wild card" compounds did not exhibit high activity, but did provide valuable insight about what would not work as a viable drug candidate. One alkyl indole aryl ether compound, **5.2**, did display good binding affinity and will likely be the subject of further attention by our group (**Chapter 5**).

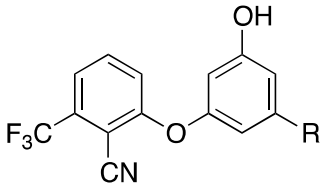
Scheme 6.1 Overview of ligand design strategy for diaryl ether cannabinoids



6.3 Results and Discussion

Biological screening of our first generation of diaryl ether cannabinoids confirmed that the novel scaffold held promise for the discovery of potent compounds. Of the compounds screened, the highest binding affinity was in approximately the low micromolar range in radioligand displacement assays. From the data illustrated in **Table 6.1**, it became clear that a hydrophobic alkyl group of three or more carbons was necessary for binding at the CB1 receptor. Compound **2.7a** bared no alkyl chain and at 1 μM concentration demonstrated little to no substitution for radioligands. Conversely, alkyl substituted compounds **2.7d,e** demonstrated clear, albeit moderate, binding affinity, and displaced more than 50% radioligand at a concentration between 1 and 10 μM. This trend was somewhat unsurprising, as hydrophobic alkyl chains are a reoccurring motif in many prototypical cannabinoid receptor ligands, such as THC (**1.16**), CP-55,940 (**1.17**), and JWH-007 (**5.1**).

Table 6.1. SAR for first generation diaryl ether cannabinoids



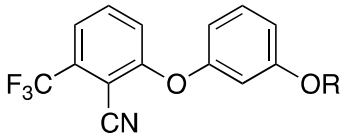
Cpd	Code	R	CB1[b] (1 μM)	CB1[b] (10 μM)	[^{3}H]SR141716A (CB1) K_i (nM)
2.7a	ARN142	H	3	37	>> 10,000[c]
2.7b	ARN156	CH_3	16	30	21,055 ± 3217[a]
2.7c	ARN167	CH_2CH_3	21	37	>> 10,000[c]
2.7d	ARN180	$CH_2CH_2CH_3$	24	60	< 10,000 [c]
2.7e	ARN191	$CH_2(CH_2)_2CH_3$	35	94	< 10,000 [c]
2.7f	ARN158	$CH_2(CH_2)_3CH_3$	39	41	293 ± 107[a]
2.7g	ARN190	$CH_2(CH_2)_4CH_3$	nd	nd	-

[a] All the values are mean ± SEM of three experiments. [b] Binding affinities at CB1 receptor measured as % inhibition. [c] Estimated K_i based on concentration range of 50% inhibition.

Given the encouraging results from our first generation compounds outlined in **Table 6.1**, a second generation of compounds was synthesized with the intent of more closely mimicking the BAY 59-3074 (**2.2**) scaffold by the introduction of alkyl ester functionality which simultaneously produced compounds with better water solubility. Also, like BAY 59-3074, second generation diaryl ethers were devoid of a phenolic hydroxyl group, though this possibly had a detrimental effect on binding affinity. Bioassay of second generation compounds is illustrated in **Table 6.2**. None of the compounds from the ester series displayed significant binding affinity. Compound **2.8e** demonstrated the most appreciable binding affinity of the ester series with an estimated modest 10μM inhibition constant. Interestingly, a notable drop-off in binding affinity occurred when the hydrophobic alkyl chain increased from five carbons to six carbons in compounds **2.8e** and **2.8f**, respectively, which is somewhat anomalous compared to trends usually observed with cannabinoids. Nevertheless, the esters tested did not possess high enough binding

affinity to warrant further study. A related compound, **2.6a**, characterized by benzyl ether functionality at the site of modification, was also tested within this series. Surprisingly, **2.6a**, with an estimated inhibition constant of approximately 1 μM, demonstrated higher binding affinity than all of the esters, **2.8b-f**, tested. Consequently, benzyl ethers built on the scaffold of **2.6a** may be the subject of future attention. Several possible conclusions may be drawn from the results of the bioassay of second generation compounds. Despite their close similarity to BAY 59-3074, alkyl esters **2.8b-f** were surprisingly devoid of activity, suggesting that electrostatic difference between an ester and a sulfonate ester was significant enough to impact binding affinity. Given that phenolic hydroxyl groups represent a common motif in many cannabinoid receptor ligands, the absence of this functionality in the compounds tested may have had a negative impact on binding affinity. Nevertheless, neither high affinity BAY 59-3074 nor moderate affinity benzyl ether **2.6a** possessed phenolic hydroxyl groups, so they may not be entirely necessary for binding affinity.

Table 6.2. SAR for second generation diaryl ether cannabinoids



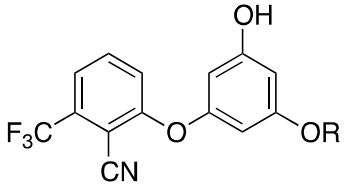
Cpd	Code	R	CB1[a] (1 μM)	CB1[a] (10 μM)
2.6a	AS109	Bn	49	56
2.8b	ARN199	$COCH_3$	18	45
2.8c	ARN200	$COCH_2CH_3$	25	34
2.8d	ARN201	$CO(CH_2)_2CH_3$	25	25
2.8e	ARN202	$CO(CH_2)_3CH_3$	25	49
2.8f	ARN203	$CO(CH_2)_4CH_3$	7	19

[a] Binding affinities at CB1 receptor measured as % inhibition.

Bioassay of representative third generation phloroglucinol derived cannabinoids is presented in **Table 6.3**. The design of the compounds in this series was based partially on first generation compounds, with the key modification being the insertion of an ether linkage where the alkyl group attached to the aromatic ring in first generation compounds. In doing so, the phenolic hydroxyl group was retained and water solubility was increased. Full binding affinity curves for compounds **4.1a**, **4.2a**, and **4.4a** were carried out (**Figure 6.2**); subsequently, precise inhibition constants were determined. Compound **4.4a** demonstrated highest binding affinity of all compounds tested, with an inhibition constant of 1.2 nM. The data demonstrated a clear trend that binding affinity increased as the length of the alkyl chain increased. Compound **4.4a**, with a six-carbon alkyl chain, demonstrated thousand-fold increased in binding affinity over the four-carbon analog, **4.2a** and the three-carbon analog **4.1a** exhibited 3-fold lower binding affinity than **4.2a**. The shape of the binding affinity curve (**Figure 6.2**) for **4.4a** (AMS-167) suggested that the compound was possibly interacting with multiple receptor sites, evidenced by the shallow

slope and plateau in the curve between 1 nM and 100 nM, however the details of this somewhat unusual curve remains yet to be fully explained.[5]

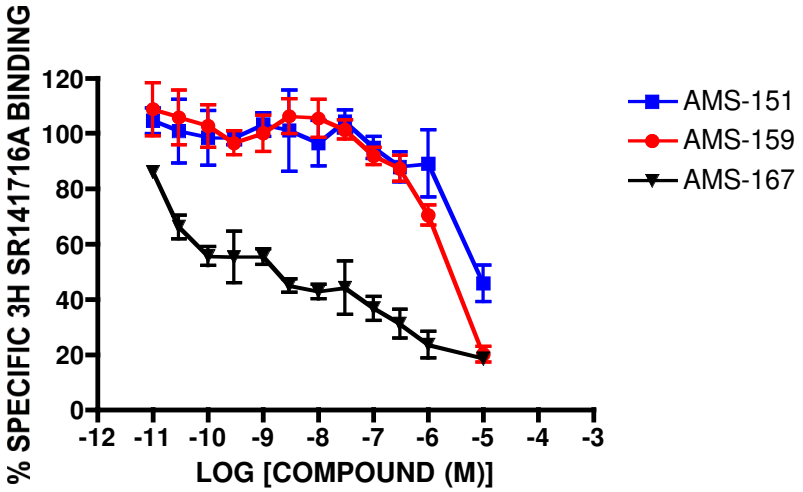
Table 6.3. Binding affinity data for third generation diaryl ether cannabinoids



Cpd	Code	R	CB1[b] (1 μM)	CB1[b] (10 μM)	[^{3}H]SR141716A (CB1) K_i (nM)
6.1	AMS137	CH_3	0	14	>> 10,000[c]
4.1a	AMS151	$CH_2CH_2CH_3$	2	59	3530 ± 976[a]
4.2a	AMS159	$CH_2(CH_2)_2CH_3$	14	78	1230 ± 125[a]
4.3a	AMS205	$CH_2(CH_2)_3CH_3$	51	52	< 1,000[c]
4.4a	AMS167	$CH_2(CH_2)_4CH_3$	65	85	1.2 ± 0.5[a]

[a] All the values are mean ± SEM of three experiments. [b] Binding affinities at CB1 receptor measured as % inhibition. [c] Estimated K_i based on concentration range of 50% inhibition.

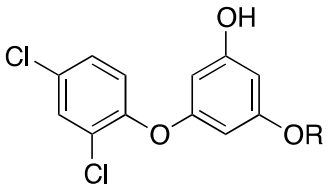
Figure 6.2. Binding affinity curves for select third generation compounds



Given the favorable binding affinity data of compound **4.4a**, the analogs outlined in **Tables 6.4**, **6.5**, and **6.6** were synthesized with the goal of exploring the impact of chlorine substitution on the otherwise experimentally untouched electron deficient aromatic ring of the diaryl ether scaffold. Aromatic dichloro-substitution patterns have been observed in some known cannabinoid receptor ligands, including Rimonabant (**2.1**), so it was possible that our new compounds would also benefit from a similar favorable interaction with the receptor. Unfortunately, the results of the biological screening were largely unsuccessful and inconclusive. The removal of the trifluoromethyl and nitrile functionality seen in previously examples negatively impacted the water solubility of the new compounds and consequently, made bioassay very difficult. Only one compound of the series, **4.8a**, was successfully screened (**Table 6.4**). With a six-carbon alkyl ether, **4.8a** was essentially the dichloro analog of potent compound **4.4a**. The analog **4.8a** demonstrated moderate binding affinity, but much lower than that of **4.4a**. Overall, diaryl ethers bearing dichloro

substitution did not represent good drug candidates and further analysis of these compounds was abandoned.

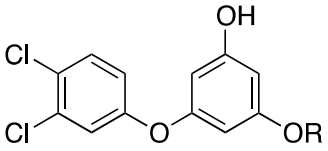
Table 6.4. Binding affinity data for third generation compounds bearing 2,4-dichloro substitution



Cpd	Code	R	CB1[a] (1 μM)	CB1[a] (10 μM)	[^{3}H]SR141716A (CB1) K_i (nM)
4.8a	AMS215	$CH_2(CH_2)_4CH_3$	51	52	< 1000[b]

[a] Binding affinities at CB1 receptor measured as % inhibition. [b] Estimated K_i based on concentration range of 50% inhibition.

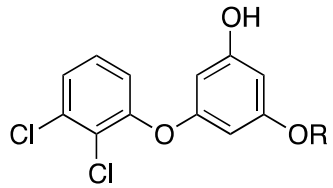
Table 6.5. Binding affinity data for third generation compounds bearing 3,4-dichloro substitution



Cpd	Code	R	CB1 (1 μM)	CB1 (10 μM)
4.9a	AMS2251	$CH_2CH_2CH_3$	IR[a]	NI[b]
4.10a	AMS2252	$CH_2(CH_2)_2CH_3$	IR[a]	NI[b]
4.11a	AMS2253	$CH_2(CH_2)_3CH_3$	IR[a]	NI[b]
4.12a	AMS2254	$CH_2(CH_2)_4CH_3$	IR[a]	NI[b]

[a] IR: Inconsistent Results [b]NI No Inhibition at 10 μM, solubility was a problem

Table 6.6. Binding affinity data for third generation compounds bearing 2,3-dichloro substitution

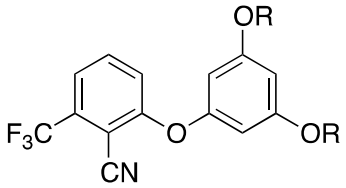


Cpd	Code	R	CB1 (1 μM)	CB1 (10 μM)
4.13a	AMS2271	$CH_2CH_2CH_3$	IR[a]	NI[b]
4.14a	AMS2272	$CH_2(CH_2)_2CH_3$	IR[a]	NI[b]
4.15a	AMS2273	$CH_2(CH_2)_3CH_3$	IR[a]	NI[b]
4.16a	AMS2274	$CH_2(CH_2)_4CH_3$	IR[a]	NI[b]

[a] IR: Inconsistent Results [b]NI No Inhibition at 10 μM, solubility was a problem

Serendipity and luck are sometimes a theme in drug design and medicinal chemistry.[6] As such, some of the synthetic by-products that were made alongside the target compounds were also screened at cannabinoid receptors for activity. A summary of these so-called "wild card" compounds is illustrated in **Table 6.7**. Unfortunately, we did not get lucky with any high affinity compounds. Nevertheless, these compounds did provide valuable insight to our structure activity relationship studies. The compounds in **Table 6.7** represent di-alkyl analogs of the very active mono-alkyl phloroglucinol-derived compounds shown in **Table 6.3**. Interestingly the di-alkyl compounds were largely devoid of activity, suggesting once again that the phenolic hydroxyl group likely plays a role in favorable interaction with the receptor. An alternative hypothesis is that these compounds were simply too lipophilic to be properly assayed, similar to the problem with the di-chloro compounds described previously.

Table 6.7. Binding affinity data for "wildcard" compounds bearing di-alkyl ether substitution patterns



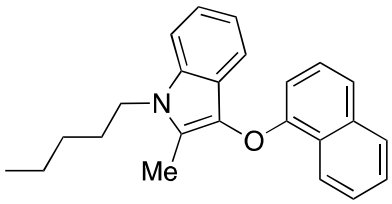
Cpd	Code	R	CB1[a] (1 μM)	CB1[a] (10 μM)	[^{3}H]SR141716A (CB1) K_i (nM)
3.6b	AMS147	H	0	19	>> 10,000[b]
3.6a	AMS115	CH_3	0	32	> 10,000[b]
4.1b	AMS150	$CH_2CH_2CH_3$	37	49	~10,000[b]
4.2b	AMS158	$CH_2(CH_2)_2CH_3$	26	42	> 10,000[b]
4.3b	AMS204	$CH_2(CH_2)_3CH_3$	nd	nd	-
4.4b	AMS166	$CH_2(CH_2)_4CH_3$	17	19	> 10,000[b]

[a] Binding affinities at CB1 receptor measured as % inhibition. [b] Estimated K_i based on concentration range of 50% inhibition.

Tangentially, a project was initiated in order to explore alkyl indole aryl ethers represented by compound **5.2**. This compound was synthesized in order to test the hypothesis that the ether analog of the known CB1 receptor ligand **5.1** would also exhibit binding affinity at the CB1 receptor (refer to Chapter 5 for further details). Though compound **5.2** seemed largely structurally distinct from our previously synthesized diaryl ethers, several common features could be noticed. Namely, both compounds consisted of two planar aromatic systems linked by an oxygen atom. Further, one of those aromatic systems was anchored to a five-carbon hydrophobic alkyl chain. Compound **5.2** was synthesized and subjected to binding affinity studies at the CB1 receptor, the results of which are illustrated in **Table 6.8** and **Figure 6.3**. With a K_i value of 246 nM, compound **5.2** demonstrated good binding affinity, however its parent analog, napthoyl indole **6.1**, had about 25-fold higher binding affinity with a K_i of 9 nM.[3] We envisage that further

refinement of this scaffold could potentially yield potent compounds. Furthermore, no efficacy data exists for alkyl indole aryl ethers like **5.2**. While parent compound **5.1** demonstrated full agonist efficacy at the CB1 receptor, it is possible that the diaryl ether analogs related to **5.2** could display diminished efficacy while retaining binding affinity. It is likely that analogs of **5.2** will be the subject of future attention.

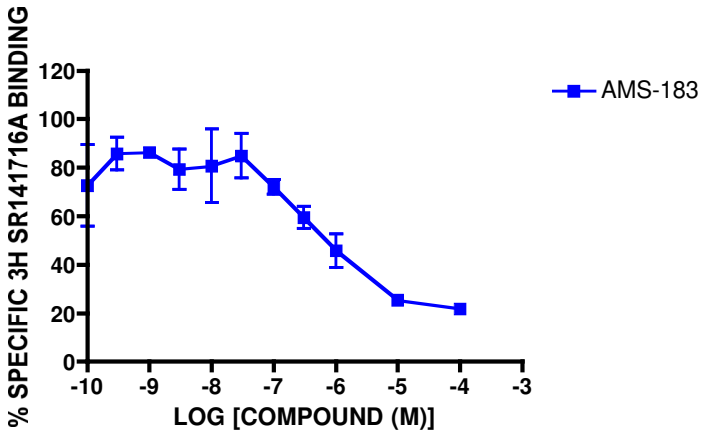
Table 6.8. Binding affinity data for novel alkyl indole aryl ether 5.2



Cpd	Code	CB1[b] (1 µM)	CB1[b] (10 µM)	[^{3}H]SR141716A (CB1) K_i (nM)[a]
5.2	AMS183	54	75	246 ± 101

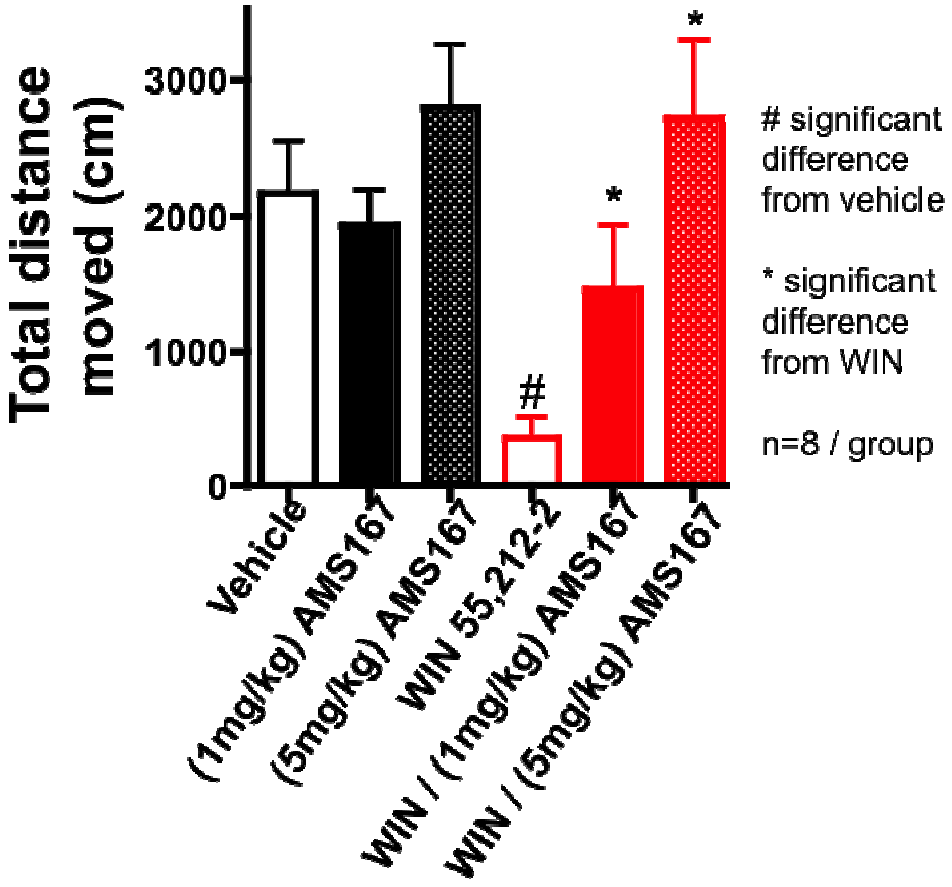
[a] All the values are mean ± SEM of three experiments. [b] Binding affinities at CB1 receptor measured as % inhibition.

Figure 6.3. Binding affinity curve for novel alkyl indole aryl ether 5.2



With its high affinity for the CB1 receptor, **4.4a** (AMS167) became our new lead compound. In order to determine if the compound had bioavailability and to make an assessment of its apparent efficacy, the compound's effect on the locomotor activity in rats was studied. It has been previously established that cannabinoid receptor agonists have a sedative effect in animal models.[7,8] Consistently, administration of the potent cannabinoid agonist WIN 55,212-2 (**5.5**) reduced the distance traveled by rats over a given time period as illustrated in **Figure 6.4**. AMS167, administered alone, did not demonstrate a significant change in locomotor activity over control at either 1 mg/kg or 5 mg/kg doses. However, when AMS167 was co-administered with WIN 55,212-2, locomotor activity was no longer attenuated as with WIN 55,212-2 alone. Furthermore, increased dosage of AMS167 relative to WIN 55,212-2 led to apparent complete blockage of its effects on locomotion by returning activity to within the same range as the control. From this study, we have concluded that **4.4a**, (AMS167) has good bioavailability and blood brain barrier penetrability as it clearly had an effect on animal behavior. The compound antagonized the effects of cannabinoid agonist WIN 55,212-2, suggesting that **4.4a** was binding to the CB1 receptor and demonstrating reduced efficacy. From this, we may conclude that **4.4a** was either behaving as a neutral antagonist or possibly an inverse agonist at CB1 receptors. Further efficacy studies will be required to determine the compounds effect on CB1 receptor second messengers. Additionally, continued animal studies are currently underway in order to determine the effect of **4.4a** on conditioned place preference in rats and will be published elsewhere**.**

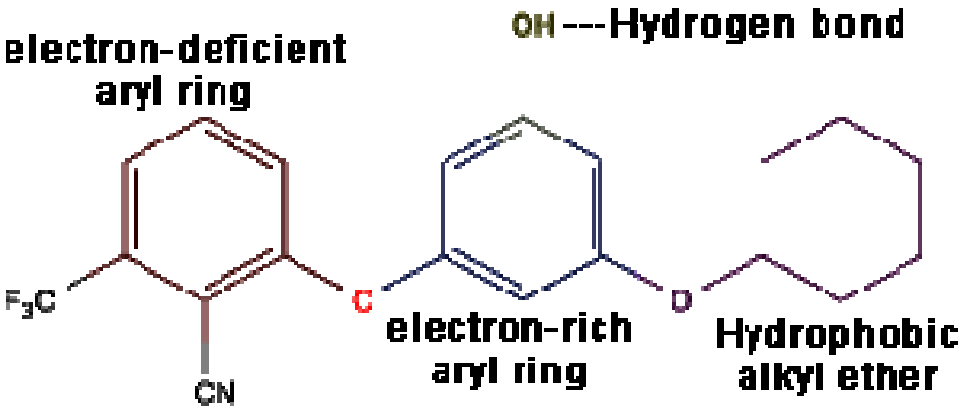
Figure 6.4. ***In vivo*** **activity of compound 4.4a (AMS167)**



In summary, with compound **4.4a** serving as our lead, a partial analysis of structure-activity for diaryl ether cannabinoids is presented in **Figure 6.5**, with the key features of the molecule highlighted. The molecule possessed an electron deficient aryl ring that was borrowed from the BAY 59-3074 scaffold. Substitution of the nitrile and trifluoromethyl groups for chlorine atoms had a negative impact on binding affinity and solubility. An electron-rich phloroglucinol-core aryl ring was connected to the electron deficient aryl ring by ether linkage. It was found that blocking the phenolic hydroxyl group had a significant negative impact on binding affinity, suggesting that hydrogen bond interaction is required for proper binding. A hydrophobic alkyl ether linkage was found to have a positive impact

on binding affinity. Further, a chain length of five to six carbons was found to produce optimal binding. Removal of the ether linkage by attaching the alkyl group directly to the electron-rich ring had a negative impact on binding. Collectively, these structural features of **4.4a** yielded a high affinity, low efficacy cannabinoid receptor ligand.

Figure 6.5. Structural features of lead compound 4.4a



6.4 Conclusion

A structure activity relationship for diaryl ether cannabinoids has been established. Through three generations of modification, several structural features have been identified on diaryl ether scaffolds that are necessary for binding affinity at the cannabinoid CB1 receptor. One high affinity compound and several moderate affinity compounds were produced. The high affinity compound represents new class of cannabinoids and is currently serving as our lead. Further pharmacological analysis of this compound will be reported elsewhere.

6.5 Acknowledgement

This research was funded by the National Institute on Drug Abuse (DA023916) and the University of New Orleans.

The biological data was obtained in collaboration with Professor Sari Izenwasser at the University of Miami Miller School of Medicine.

6.6 Experimental Section

CB1 binding assay. Cerebellum was dissected on ice and suspended in 10 volumes of ice-cold buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.1 M EDTA, pH 7.4) and homogenized for 20 sec with a polytron (setting 6). The homogenates were centrifuged at 31000 x *g* for 10 min at 4 °C, the supernatants discarded and the tissue resuspended in buffer and centrifuged again. The final pellet was resuspended at a concentration of 5 mg wet weight/mL. Triplicate samples of membranes were incubated in buffer for 2 h at 30 °C in a final volume of 1 mL buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 100 mM NaCl, pH 7.7 with NaOH) in the presence of 0.5 nM [^{3}H]SR 141716A and 30 μM GDP. Non-specific binding was determined as binding in the presence of 1 μM CP 55,940. The incubation was terminated by rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in cold Tris buffer). The filters were rinsed 3 times, each with 5 mL Tris buffer containing 0.1% BSA and transferred to scintillation vials. Beckman Ready Value Scintillation Cocktail was added to the vials, which were counted the next day at an efficiency of approximately 40%.

***In vivo* in locomotor activity studies.** Compounds were tested for their ability to antagonize the locomotor decreasing effects of WIN 55212-2. Male Sprague-Dawley rats (Charles River, Wilmington, MA) were used for all studies. Rats were housed two per cage in a temperature and humidity-controlled environment under a 12 h light/dark cycle. Food and water were available *ad libitum*. Rats were placed in 40.4 x 40.5 x 30.3 cm Plexiglas enclosures placed in Digiscan locomotor activity monitors (Omnitech Electronics, Columbus, OH). Panels of infrared beams (16 beams per side) with corresponding photodetectors were located on the sides of the chambers, and distance traveled, in centimeters, and horizontal activity (expressed as number of beam breaks) was measured in 5 min intervals for 60 min post-injection, as described previously.[9] Each compound was tested with 3 mg/kg WIN 55212-2, to determine whether there are any antagonist effects. Initial doses will be determined by comparing the binding affinity of the new compound to that of known compounds such as □^{9}-THC and CP 55,940, or the appropriate parent compound. Each dose was administered i.p. in a volume of 1 ml/kg. Time course data was analyzed by ANOVA comparing the effects of the test compound to vehicle across time. A one-way ANOVA using dose as a variable was used to determine the relationship between the antagonist and blockade of the locomotor-decreasing effects of WIN 55,212-2. *P* values less than 0.05 were considered significant. There were 8 rats/condition for the locomotor activity studies.

6.7 References

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7

Gram-scale Preparation of AMS-167, A Novel Low Efficacy Cannabinoid Receptor Ligand

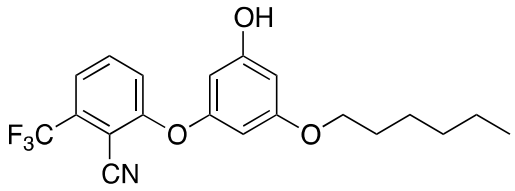
7.1 Abstract

In vitro assay for Compound **4.4a** (AMS167) appeared to be very promising regarding its utility as a high affinity low efficacy cannabinoid receptor ligand. As such, larger-scale preparations were warranted in order to provide sufficient material for further study. Our previously established methods were modified and optimized accordingly and a 1.6 g batch of compound AMS167 was synthesized in 34 % yield overall in three steps.

7.2 Introduction

As a result of the structure activity relationship studies outlined in **Chapters 2-5**, AMS167 emerged as a promising lead compound with binding affinity of 1.2 nM and an apparent neutral agonist efficacy profile at the CB1 receptor *in vitro*. Animal studies also supported the indication that the compound behaved as a neutral antagonist *in vivo*. Additional pharmacology and experiments in animal studies required gram-scale quantities of AMS167, so our previous synthetic methods were adjusted accordingly. Only slight modification was required in order to optimize our previously described method for the production of this compound on a gram-scale.[1] In doing so, the cost of the reaction was reduced and the yield was improved.

Figure 7.1. AMS167, a novel high affinity cannabinoid neutral antagonist

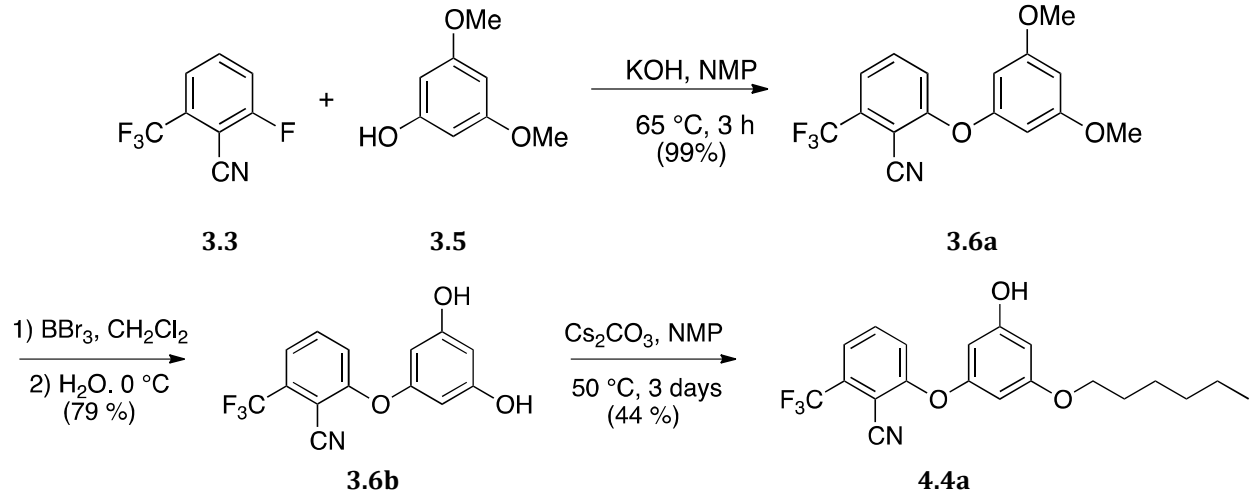


4.4a

7.3 Results and Discussion

The nucleophilic aromatic substitution (S_NAr) reaction to couple aryl fluoride **3.3** to dimethoxyphenol **3.5** proceeded as previously described except potassium hydroxide was used to afford the phenoxide intermediate as opposed to cesium carbonate. This was mainly due to the fact that there wasn't enough cesium carbonate on hand at the time of the syntheses. Interestingly, this substitution had no effect on the reaction yield and product recovery was nearly quantitative. Fortuitously, the substitution also reduced the cost of the reaction, as cesium carbonate is approximately 3-5x more expensive than potassium hydroxide. In an afternoon of work, it has been demonstrated that the reaction can produce up to 16 g of product **3.6a** in sufficient purity to be used for further synthesis.

Scheme 7.1. Synthesis of AMS167



Boron tribromide promoted demethylation of **3.6a** to afford the diol **3.6b** was accomplished by the procedure described by **Chapter 3**. This reaction has been demonstrated at several scales and results are fairly reproducible with linear scaling of reagents. It was not surprising that the demethylation step was affected by the same previously observed side reaction and gave a mixture of side products that made it necessary to purify the phloroglucinol derivative **3.6b** by column chromatography. It is suspected, but not confirmed, that boron tribromide undergoes an unfavorable reaction with the trifluoromethyl group on **3.6** given a previous report that trifluoromethylarenes were converted to tribromomethylarenes using boron tribromide in carbon tetrachloride.[2] Nevertheless, the synthesis of up to 3 g of **3.6b** was relatively straightforward. It is suspected that this reaction would work on scales larger than those presented here, however working with large quantities of neat boron tribromide is generally undesirable.

As described previously in **Chapter 4**, multiple sites of reactivity and complex reaction kinetics affect the alkylation of phloroglucinol derivative **3.6b** to produce the desired mono-alkylated product **4.4a.** The milligram-scale synthesis of **4.4a** was modified

slightly and scaled accordingly, resulting in an improvement of product recovery from 34 % to 44 %. It was hypothesized that an overall excess of the diol **3.6b** relative to base and alkyl halide would favor mono-alkylated product. Reagent quantities were controlled such that the diol was initially in 2.6-fold molar excess of cesium carbonate and 2.3-fold molar excess of iodohexane relative to base. The reaction was carried out at a lower temperature of 50 °C compared to 70 °C previously and for a longer amount of time in an effort to reduce side reactions such as C-alkylation. However, as a result of the longer reaction time, it became evident that the somewhat volatile iodohexane had begun to collect on the walls of the condenser. An additional small amount of iodohexane was added after 48 h in order to compensate for this apparent loss. Given the complex kinetics of this reaction and that several variables were modified at once in the scaled procedure, it would be difficult to conclude that any one factor improved the yield. The only conclusion is that the yield improved, and that is good. Furthermore, this reaction has been repeated using similar conditions at the same scale with about the same product recovery.

Purification of the crude reaction mixture was straightforward. Using flash column chromatography with an increasing gradient of ethyl acetate in hexanes, dialkyl ether **4.4b** eluted first, followed by AMS167, and finally about 30 % of unreacted starting material **3.6b** was recovered for recycling. While the solid white AMS167 isolated from the column was of sufficient purity to be used to further study, if desired, the compound could be recrystallized overnight from hexanes with about 80% recovery of the crystalline solid.

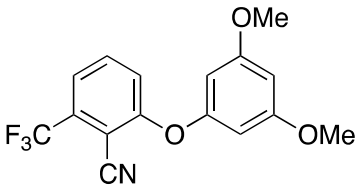
7.4 Conclusion

In order to provide a sufficient amount of our promising lead in a cannabinoid receptor ligand study, a scaled reaction procedure was developed. It has been demonstrated that 1 - 2 grams of AMS167 can be synthesized in 34% yield in three steps using simple reaction conditions in a few days at low cost.

7.5 Acknowledgement

This research was funded by the National Institute on Drug Abuse (DA023916) and the University of New Orleans.

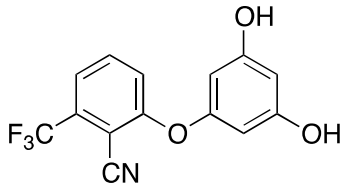
7.6 Experimental Section



2-(3,5-dimethoxyphenoxy)-6-(trifluoromethyl)benzonitrile (3.6a)

Dimethoxyphenol **3.5** (8.5 g, 55 mmol) was dissolved in NMP (30mL). Potassium hydroxide (4.4 g, 66 mmol based on 85% purity) was crushed to a fine powder with mortar and pestle and then added at once to the phenol solution. The suspension was stirred and heated gently for 10 minutes resulting in a grey-green colored phenoxide solution and some undissolved KOH. To the warm suspension, 2-fluoro-6-trifluoromethylbenzonitrile **3.3** (9.5 g, 50 mmol) was added dropwise (liquefied by gentle warming with heat gun). The reaction was allowed to stir at 65 °C for 3 h, resulting in a granular white solid suspended in brown liquid. TLC (70% hexanes/EtOAc) indicated complete disappearance of 2-fluoro-

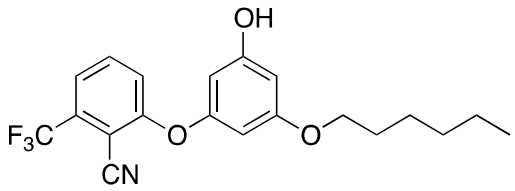
6-trifluoromethylbenzonitrile. The suspension was brought to room temperature and water (100mL) followed by toluene (100mL) was added. The toluene was collected and the brown aqueous layer was extracted with portions of toluene (2 x 100 mL). Pooled organic extracts were washed with 1N NaOH (1N, 100 mL), water (100 mL), brine (100 mL) then dried over sodium sulfate. Removal of solvent under reduced pressure on a rotoevaporatoar resulted in an off-white waxy solid, to which near-boiling water (60 mL) was added. The gooey, inhomogenous mixture was stirred vigorously overnight. The white solid was collected and washed with the aid of additional water. After drying completely under high vacuum **3.6a** (16 g, 99%) as a shiny white solid was isolated, mp 95-97 °C: ^{1}H NMR ($CDCl_3$; 400MHz) δ: 7.57 (t, J = 8.0 Hz, 1H) 7.44 (d, J = 8.0 Hz, 1H) 7.13 (d, J = 8.0 Hz, 1H) 6.36 (s, 1H) 6.25(s, 2H) 3.78 (s, 6H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 162.2, 161.5, 156.1, 134.4, 134.1, 123.8, 121.1, 120.4, 112.6, 100.8, 98.9, 97.9, 55.7.



2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile(3.6b)

A solution of **3.6a** (6.5 g, 20 mmol) in dry methylene chloride (30 mL) in an oven-dried flask under a nitrogen atmosphere was cooled on ice. Neat boron tribromide (8mL, ~80 mmol) was carefully drawn into a syringe and added via septum to the cold aryl methyl ether solution over about 15 minutes. The orange colored solution was allowed to come to room temperature slowly overnight. The reaction mixture was carefully added to a pressure-equalized addition funnel. Methylene chloride (30 mL) was used to rinse the flask

and subsequently dilute the reaction mixture in the addition funnel. The addition funnel was placed over a flask containing vigorously stirring cold water (150 mL) and crushed ice. The orange boronate-complex solution was added dropwise over about 30 minutes, adding additional ice to the flask if necessary. The mixture was then allowed to stir for one hour to complete the hydrolysis. The very acidic aqueous suspension was neutralized (pH 6 - 7) with NaOH (2N) then extracted with EtOAc (3 x 150 mL). Pooled organic extracts were washed with water then brine and dried over sodium sulfate. The extracts were concentrated under reduced pressure on a rotoevaporatoar to furnish an orange oil. Silica was added to the crude residue and it was dry-loaded onto a silica column and purified by flash chromatography using 60:40 EtOAc:hexanes. The product eluted just before a visible orange band on the column. Pooled fractions were concentrated to afford a light yellow oil that trapped EtOAc. Product spontaneously crystallized with the addition of several milliliters of hexanes to give **3.6b** (4.65 g, 79%) as a white solid, mp 178 – 180 °C: ^{1}H NMR (400MHz; DMSO-d_6) δ: 9.66 (s, 2H) 7.81(t, J = 8.0 Hz, 1H) 7.62(d, J = 8.0 Hz, 1H) 7.31(d, J = 8.0 Hz, 1H) 6.17(s, 1H) 6.00(s, 2H). ^{13}C NMR (100 MHz; DMSO-d_6) δ: 161.5, 160.5, 156.5, 136.2, 132.7, 124.3, 122.3, 121.4, 113,2, 100.7, 100.0, 98.7. Anal. Calcd for $C_{14}H_8F_3NO_3$: C, 56.96; H, 2.73; N, 4.74. Found: C, 56.90; H, 2.83; N, 4.54.



2-(3-(hexyloxy)-5-hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (4.4a)

To a stirring solution of **3.6b** (4.00 g, 13.5 mmol) in NMP (100 mL) was added Cs_2CO_3 (1.7 g, 5.2 mmol) and the reaction mixture was brought to 50 °C. 1-Iodohexane (2.3 g, 11 mmol) was added via syringe over 1h. The reaction stirred for 48 h at this temperature then an additional (0.2 g, 0.9 mmol) was added and the reaction stirred for an additional 24 h. The tan colored suspension was brought to room temperature and dumped into ice water (100 mL). The mixture was brought to neutral pH by dropwise addition of HCl (6N, ~2 mL) that evolved CO_2 gas and precipitated a brown oil. The aqueous mixture was extracted Et_2O (3 x 75 mL). Pooled organic layers were washed once with H_2O (150 mL), brine (150 mL) then dried over Na_2SO_4 and concentrated. The resulting oily tan residue was subjected to SiO_2 column chromatography 1:9 EtOAc:Hexanes which eluted dialkyl ether **4.4b** (1.2 g, 28%), mp 30-32 °C. Continued chromatography with 2:8 EtOAc:Hexanes eluted the desired monoalkyl ether AMS167, **4.4a** (1.56 g, 44 %). The white solid was recrystallized from hexanes; mp 80-82 °C: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.58 (t, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 6.30 (t, *J* = 2.0 Hz, 1H), 6.21 (t, *J* = 2.0 Hz, 1H), 6.19 (t, *J* = 2.0 Hz, 1H), 5.40 (s, 1H), 3.90 (t, *J* = 6.6 Hz, 2H), 1.75 (quintet, *J* = 7.3 Hz, 2H), 1.47-1.39 (m, 2H), 1.36-1.30 (m, 4H), 0.90 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ: 161.8, 161.6, 158.5, 156.1, 134.6, 134.3, 123.7, 121.0, 120.4, 120.3(q, 5Hz), 112.6, 100.3, 99.8, 99.4, 68.7, 31.7, 29.2, 25.8, 22.8, 14.2.

Continued chromatography with 6:4 EtOAc:Hexanes led to recovery of the diol starting material **3.6b** (1.2 g, 30 %).

7.7 References

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8

Synthesis of Aminobenzocyclobutenes Towards the Development of New Monoamine Receptor Ligands

8.1 Abstract

Aminobenzocyclobutenes (ABCBs) represent a medicinally unexplored class of molecules that exhibit the potential to provide valuable insight about the biogenic amine neurotransmitter pharmacophore and provide potential treatments for central nervous system disorders. A novel synthetic method has been explored in order to develop a general process for the synthesis of rigid ABCB analogs of known phenethylamines (PEAs). A key intermediate was determined to be 1-cyanobenzocyclobutene. Several routes to this compound were explored and subsequently an optimized procedure was developed reliably providing this compound in 65% yield. Additional reactions were attempted to produce phenyl substituted 1-cyanobenzocyclobutenes with mixed success. Using the synthetic approach devised here, from 1-cyanobenzocyclobutene, four novel ABCB isomeric analogs of a known pharmacologically active PEA (RS)-phenylpropan-amine were synthesized.

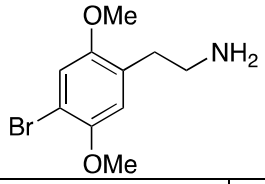
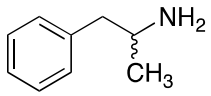
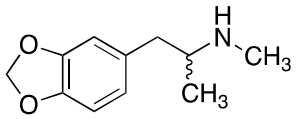
Scheme 8.1. Conformational restriction of ethylamine sidechain in phenethylamines

flexible phenethylamine **rigid ABCB analog**

8.2 Introduction

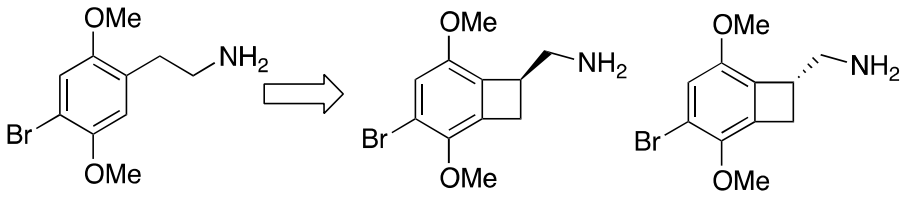
Substituted phenethylamines represent a structurally simple yet pharmacologically diverse set of molecules and, based on the corresponding molecular substitutions, exhibit a range of affinities and efficacies upon different sites within the body and especially the CNS. Endogenous phenethylamine neurotransmitters include dopamine, epinephrine and norepinephrine. Pharmacologically active exogenous PEAs typically elicit their effects by the manipulation of one or more of the biogenic amine neurotransmitter pathways: serotonin, dopamine and/or norepinephrine. Based partially on the extent to which they interact with each of these pathways, the physiological effects of substituted PEAs broadly fall under three descriptions: stimulants, psychedelics and/or entactogens. Stimulants exert their effects predominantly through the facilitation of norepinephrine and/or dopamine activity.[1] Psychedelic activity is nearly always correlated with agonism of the serotonin $5HT_{2A}$ receptor, though concurrent interaction with dopamine and norepinephrine is also typical.[2] The pharmacology of entactogens is slightly less clear, though common features include serotonin-releasing ability in addition to dopaminergic activity. Entactogens produce subjective physiological effects that resemble, but are fundamentally distinct from those of psychedelics or stimulants.[3] Some prototypical PEAs and their general descriptor class are outlined in **Figure 8.1**.

Figure 8.1. Prototypical substituted phenethylamines

PEA			
Common name	2C-B	Amphetamine	MDMA
Class	psychedelic	stimulant	entactogen
Code	**8.1**	**8.2**	**8.3**

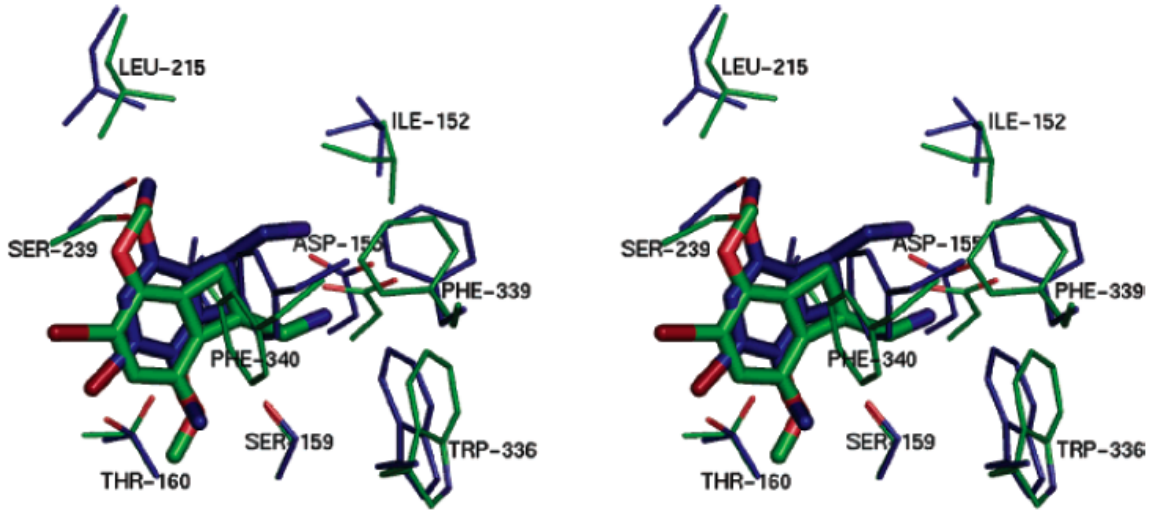
It was recently hypothesized and subsequently demonstrated by Nichols *et al.* that conformational restriction of the ethylamine side chain in prototypical $5HT_{2A}$ agonist 2C-B **(8.1)** to produce ABCB **8.4** had a direct effect on ligand binding affinity and its ability to activate downstream signaling pathways at the $5HT_{2A}$ receptor.[4] *In silico* docking simulations of **8.1** and the corresponding ABCB **8.4** within a homology model of the $5HT_{2A}$ receptor (**Figure 8.2**) predicted this activity and specifically that that enantiomer **8.4a** would direct the protonated amine towards ASP-156 within the receptor leading to a key ionic interaction required for binding, suggesting that **8.4a** would have very high binding affinity. To test this theoretical model, compounds **8.4a** and **8.4b** were synthesized and evaluated for activity *in vitro* and *in vivo*. The hypothesis was well supported by the data; **8.4a** demonstrated picomolar affinity for $5HT_{2A}$ and enantiomer **8.4b** had a K_i value in the low nanomolar range, slightly above the binding affinity for parent compound **8.1**.

Scheme 8.2. Aminobenzocyclobutene analogs of 2C-B



8.1 **8.4a** **8.4b**

Figure 8.2 Cross-eyed stereo overlay of compounds 8.4a (blue) and 8.4b (green) docked in within the putative binding site of a homology model of the $5HT_{2A}$ receptor.[4,5]

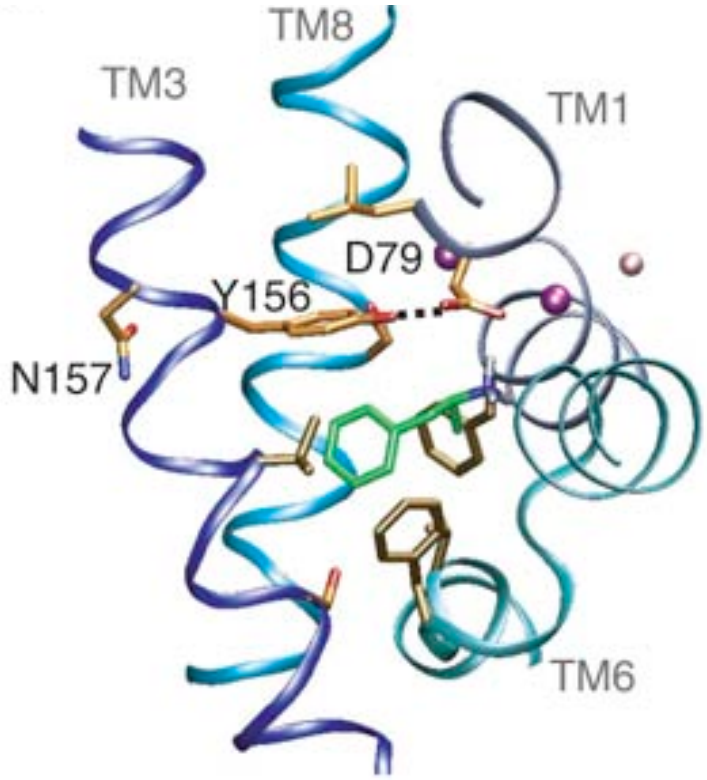


Perhaps more compelling, the experimental efficacy data suggested that these conformationally-restricted phenethylamines expressed functional selectivity depending on which stereoisomer was tested, meaning they were able to differentially stimulate the activation of several downstream signaling pathways: phospholipase C-mediated inositol triphosphate turnover compared to arachidonic acid and production of endocannabinoid 2-arachidonyl glycerol.[6] Most data regarding ligand interaction with serotonergic systems comes from phospholipase C (PLC)-mediated responses. The concept of functional selectivity is an emerging concept in pharmacology and research suggests that subtle

changes in ligand structure results in the activation of a multitude of downstream signaling pathways.[6,7] Further, these subtle variations in ligand structure have significant impacts on overall *in vivo* activity of the drugs.[8] Our understanding of the connection between the ligand/receptor interaction and *in vivo* activity is not well established.

In light of the interesting results presented above, the research described in this chapter was set in motion with the ultimate goal of exploring the synthesis and pharmacology for ABCB analogs of the other two general classes of PEAs, the stimulants and entactogens, represented by prototypical compounds **8.2** and **8.3**, respectively. We envisage that these compounds could help verify several hypotheses. In the same way that optimal binding is achieved in $5HT_{2A}$ receptor when the protonated ethylamine side chains of PEAs are held outside of the plane of the aromatic ring, ABCB analogs of prototypical stimulants and entactogens **8.2** and **8.3** would demonstrate that the optimal binding orientation prefers similar placement of the protonated amine at the active sites of other targets within the CNS including, but not limited to dopamine, serotonin and norepinephrine receptors and/or transporters. In support of this hypothesis, a recent study of the stimulant **8.2** docked at the binding site of the dopamine transporter appears to demonstrate this preferred out-of-plane conformation (**Figure 8.4**).[9] Second, the rigid ABCB analogs would exhibit greater functional selectivity at receptor sites compared to their flexible parent compounds. Given that the binding of rigid compounds could bias receptor towards fewer conformational microstates, it is possible that the rigid compounds would differentially affect second messenger pathways for a single receptor compared to the flexible analog. [10]

Figure 8.3. Amphetamine, 8.2, (green) docked within the binding site of the dopamine transporter[9]



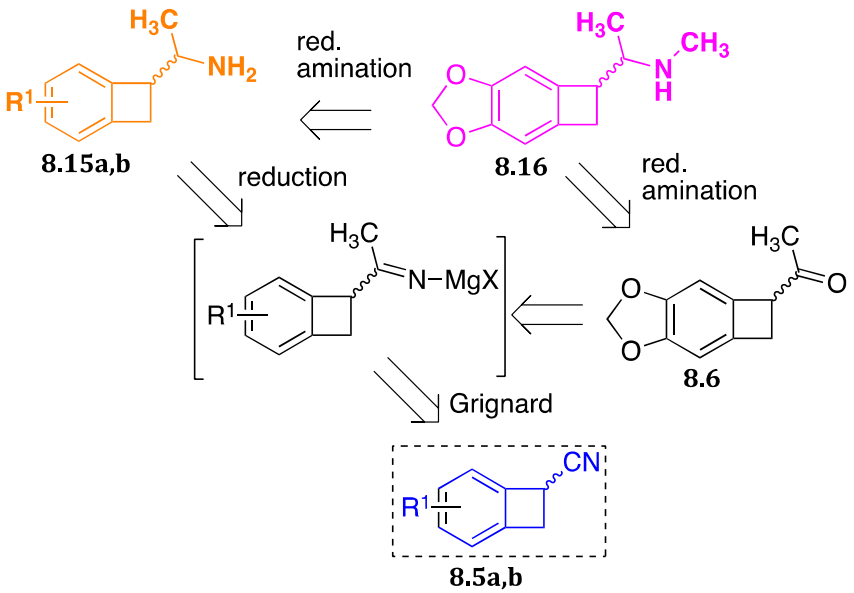
Rigid ABCB analogs of PEAs will provide important probes capable of furthering our understanding of multiple receptor pharmacophores. These compounds will be useful to scientists in a number of fields including: computational chemists studying *in silico* homology models of the receptors, molecular biologists interested in the molecular signaling cascade involved in GPCR-mediated neurotransmission, x-ray crystallographers comparing the crystal structures of rigid and flexible analogs, synthetic organic chemists interested in benzocyclobutane synthesis, and medicinal chemists developing medications for treating psychological disorders.

With the above rationale and hypotheses in mind, we set forth to develop a robust synthetic methodology capable of producing the ABCB analogs of PEAs **8.2** and **8.3** from readily available inexpensive starting materials.

8.3 Results and Discussion

Retrosynthetic analysis of the target scaffolds illustrated in **Scheme 8.3** indicated that 1-cyanobenzocyclobutene derivatives **8.5a** and **8.5b** could potentially be key intermediates. Using a parallel synthetic approach, virtually any ABCB PEA analog, including the target compounds **8.15a** and **8.16**, could potentially be synthesized from derivatives of this versatile substrate. ABCBs bearing alpha-methyl functionality, as in **8.15a,b**, could be produced by a Grignard mediated process from **8.5a,b** followed by direct reduction of the intermediate imine to the corresponding amine. Alternatively, hydrolysis of the intermediate imine would afford ketone **8.6**, precursor to target compound **8.16,** which could undergo reductive amination with the appropriate (alkyl)amine. A second approach to **8.16** could involve reductive amination on **8.15b** using an appropriate carbonyl compound.

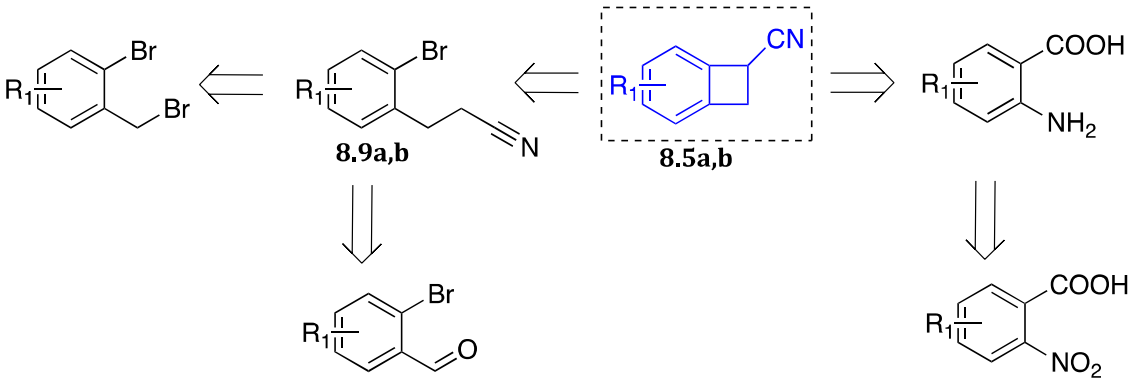
Scheme 8.3. Retrosynthetic analysis to target compounds from key intermediates 8.5a,b



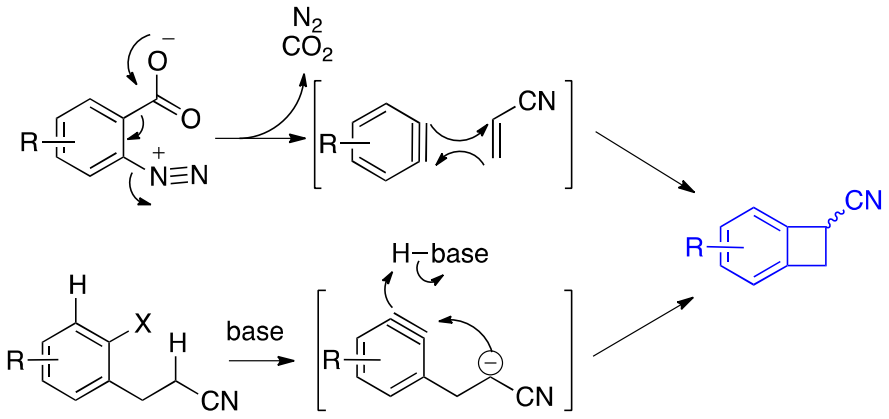
For suffixes a,b: R^1 = H or methylenedioxy, respectively.

The retrosynthetic strategy to key intermediates **8.5a,b** from commercially available starting materials is illustrated in **Scheme 8.4**. Both of the imagined routes relied on the formation of reactive aryne intermediates to form the strained 4-membered carbocycle, the corresponding mechanisms are illustrated in **Scheme 8.5**. From 2-bromohydrocinnamonitriles **8.9a,b**, strongly basic conditions simultaneously generate the aryne and deprotonate the relatively acidic alpha proton with subsequent regioselective cyclization to 1-cyanobenzocyclobutenes **8.5a,b**. Alternatively, zwitterionic benzenediazonium-2-carboxylates liberate gaseous nitrogen and carbon dioxide upon heating to generate arynes, which undergo [2+2] cycloadditions with acrylonitrile to form 1-cyanobenzocyclobutenes.

Scheme 8.4. Retrosynthetic strategy 8.5a,b from commercially available starting materials

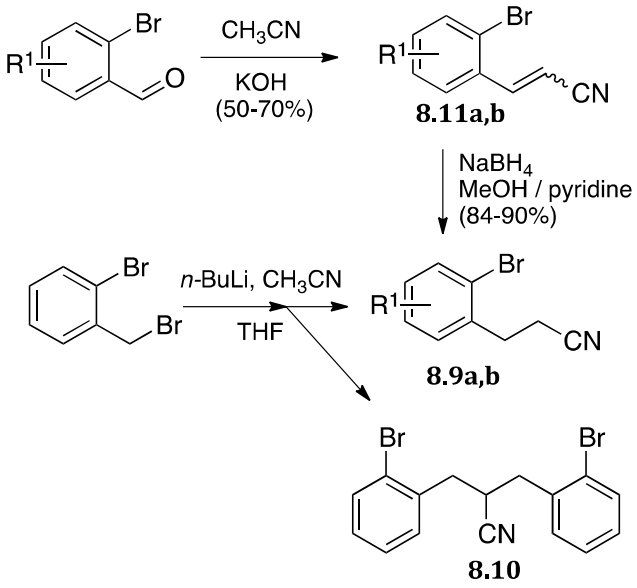


Scheme 8.5. Mechanisms to 1-cyanobenzocyclobutenes



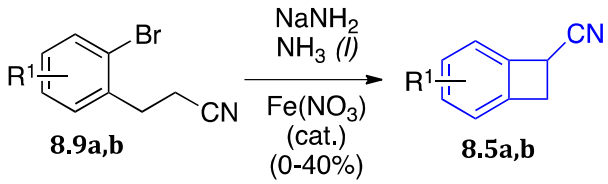
Both synthetic approaches illustrated by the retrosynthesis in **Scheme 8.4** to precursor 2-bromohydrocinnamonitriles **8.9a,b** were attempted with mixed success **(Scheme 8.6)**. Initially, the S_N2 route from bromobenzyl bromide seemed most attractive as it could produce the desired 2-bromohydrocinnamonitriles in one synthetic step. Unfortunately, using the conditions shown in **Scheme 8.6** produced a mixture of the desired product and compound **8.10**, with separation of the two being quite difficult using conventional techniques. Instead, a two-step procedure from commercially available 2-bromobenzaldehydes proved to be more reliable. 2-bromocinnamonitriles **8.10a,b** were produced in good yield by condensation of the corresponding aldehydes with acetonitrile under alkaline conditions based on the procedure described by Gokel.[11] The reduction of the unsaturated nitriles in methanolic pyridine to **8.9a,b** was straightforward and has been described elsewhere.[12]

Scheme 8.6. Attempted synthetic routes to 2-bromohydrocinnamonitriles 8.9a,b



The cyclization of 2-bromohydrocinnamonitriles to 1-cyanobenzocyclobutenes using sodium amide in liquid ammonia has been described elsewhere.[13] As illustrated in **Scheme 8.7**, using a similar approach, key intermediates **8.5a,b** were afforded in poor to moderate yield. Sodium amide was generated *in situ* by the addition of sodium metal to liquid ammonia. It is worth noting that the success of this reaction was highly dependent upon conditions being kept absolutely anhydrous throughout the course of the reaction.

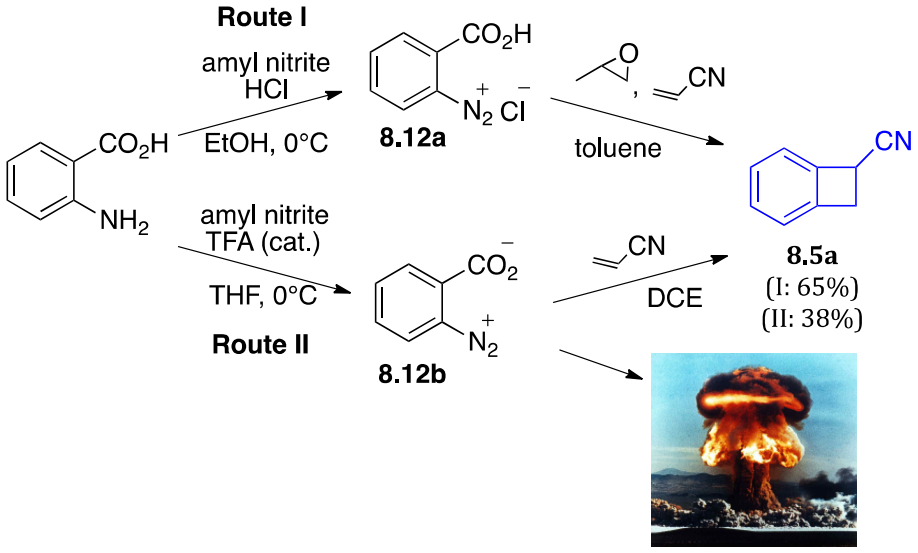
Scheme 8.7. Cyclization of 2-bromohydrocinnamonitriles using sodium amide in liquid ammonia



Though target intermediates **8.5a,b** were synthesized using the procedure outlined in **Scheme 8.7**, the apparent moisture sensitivity and handling of liquid ammonia led to a cumbersome and tedious procedure that yielded an unsatisfactory amount of product for

the work involved. Therefore, an alternative approach to 1-cyanobenzocyclobutenes was explored. Previous reports have shown that 1-cyanobenzocyclobutene may be produced from the diazotization product of anthranilic acid followed by cyclization with acrylonitrile.[4] We explored two possible variations of this procedure, as described in **Scheme 8.8.** Route II relied on a previously established method using catalytic amounts of trifluoroacetic acid and produced the corresponding zwitterion **8.12b** directly, which cyclized to **8.5a** in DCE with a stoichiometric amount of acrylonitrile in moderate yield.[14] The major drawback of Route II was the explosive nature of compound **8.12b**. For safety, the product must remain wet, and it is not recommended that the procedure be carried out on a scale larger than a few grams.

Scheme 8.8. 1-Cyanobenzocyclobutene from anthranilic acid

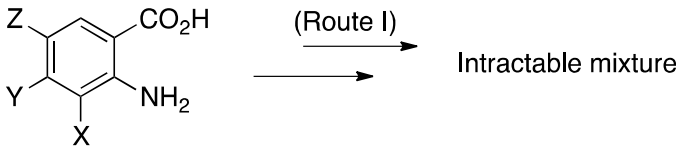


Route I represented a more traditional diazotization procedure and resulted in a more stable (not so explosive) diazonium salt. The required zwitterion was generated *in*

situ using propylene oxide as an HCl scavenger during the cyclization reaction. Several sets of reaction conditions and solvent systems were evaluated for the cyclization of **8.12a** to **8.5a**: the solvents, DCE (worked, low yield), NMP (unsuccessful, instantly turned to hot red gelatinous mess), THF (worked, low yield), excess acrylonitrile as solvent (worked, ~40 % yield), toluene (worked well), and benzene (worked well) were evaluated. Neither stirring the reaction at room temperature overnight nor immersion in an ultrasonic bath caused significant improvement in product recovery. It was found that gentle heating at 40-50 °C for 4-5h provided the best results and minimal side products as evidenced by TLC. To summarize, gently heating **8.12a** in toluene or benzene with propylene oxide and excess acrylonitrile for 4 hours reliably produced **8.5a** in 60-65% yield. It's worth noting that this is a significant improvement over similar methods to benzocyclobutenes and is, to our knowledge, the first reported use of aromatic solvents systems for [2+2] cycloadditions to 1-cyanobenzocyclobutenes.

A side project with the goal of exploring the regioselectivity and overall generality of Route I in **Scheme 8.8** was initiated; the same conditions were applied to substituted anthranilic acids **8.13** and **8.14** in **Scheme 8.9**. Unfortunately, neither of the attempted reactions produced acceptable results. Unexpectedly, neither electron deficient (**8.13**) nor electron rich (**8.14**) anthranilic acids underwent the cyclization cleanly. Therefore, for certain substituted PEA analogs, the cyclization route shown in **Scheme 8.7** might be a better option.

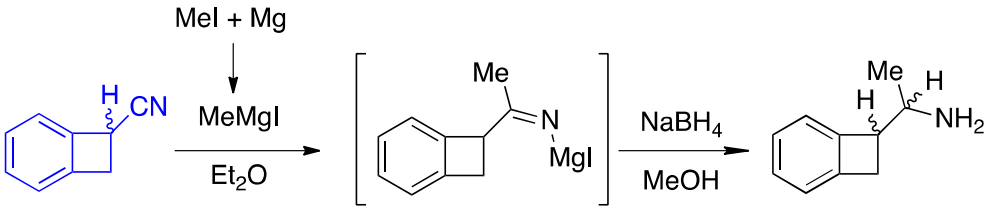
Scheme 8.9. Unsuccessful attempts at [2+2] cycloadditions on substituted anthranilic acids



8.13: X = Y = H; Z = NO_2
8.14: X = Y = Z = OMe

With the success of the reaction shown in **Scheme 8.8,** Route I, large amounts of **8.5a** could be synthesized quickly and reliably, and attention was focused on exploring the final reactions required to produce the target compound **8.15a**. Fortunately, a report existed describing an applicable procedure, and so our method was based on the same protocol.[15] Briefly, methylmagnesium iodide in dry ether reacted with **8.5a** to form an intermediate imine that was directly reduced using sodium borohydride in methanol (**Scheme 8.10**). However, unlike parent compound **8.2** with only one stereocenter and usually exists as a racemic mixture, compound **8.15a** possessed two stereocenters and therefore, four distinct compounds were possible—two pairs of enantiomers that were diastereomers (**Scheme 8.11**).

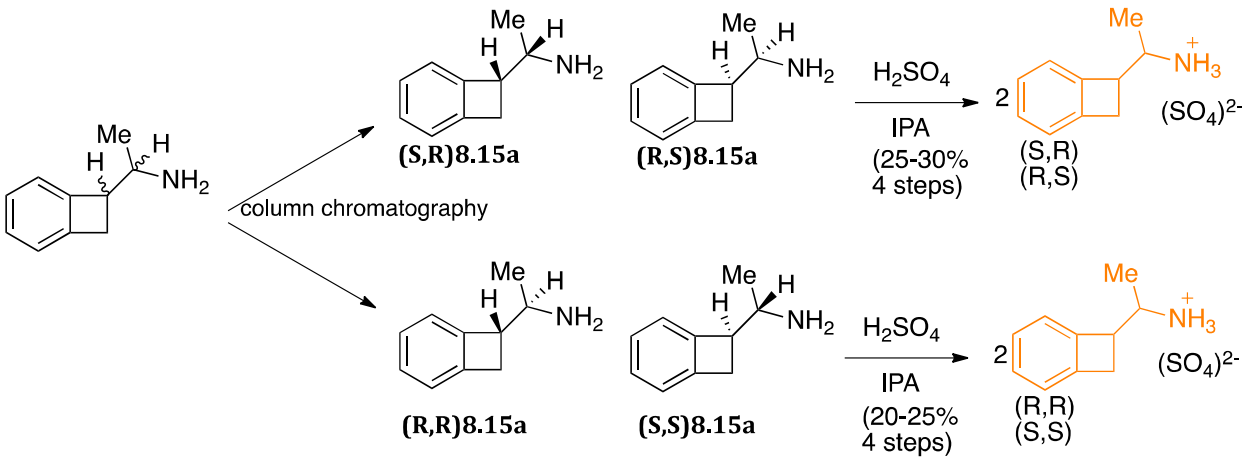
Scheme 8.10. Grignard-mediated methylation and subsequent reduction of 8.5a



8.5a **8.15a**

The resulting diastereomers of **8.15a** were resolved by careful column chromatography on silica. We found that 2% methanol in methylene chloride with a few drops of ammonium hydroxide as a modifier worked as an acceptable mobile phase, though some overlap of product fractions was typical. The chromatography was quite tedious and time consuming and the exploration of alternative methods for the purification of these compounds is recommended. Perhaps derivation of the amine, such as Boc protection, prior to chromatography would facilitate the separation.

Scheme 8.11. Resolution of 8.15a diastereomers



Each pair of enantiomeric **8.15a** freebases (of questionable stability) was immediately precipitated as the corresponding sulfate salts by the addition of concentrated sulfuric acid to a solution of each in dry isopropanol.

Upon comparison of the proton NMR spectra for the diastereomers, subtle differences in shifts were observed (**Figure 8.4**), however the data was not sufficient to completely characterize the compounds. For conclusive identification of the absolute stereochemistry for each pair of enantiomers isolated, an X-ray crystal structure was desired. We found that the sulfate salts of **8.15a** recrystallized well from 10% water in

ethanol. The X-ray crystal structure for the pair of enantiomers that eluted from the column first (lower relative polarity) is shown in **Figure 8.5**, and proved to be the (S,R)/(R,S) enantiomeric pair.

Figure 8.4. Overlayed ^{1}H NMR spectra for 8.15a diastereomers, (RS/SR) red, RR/SS blue

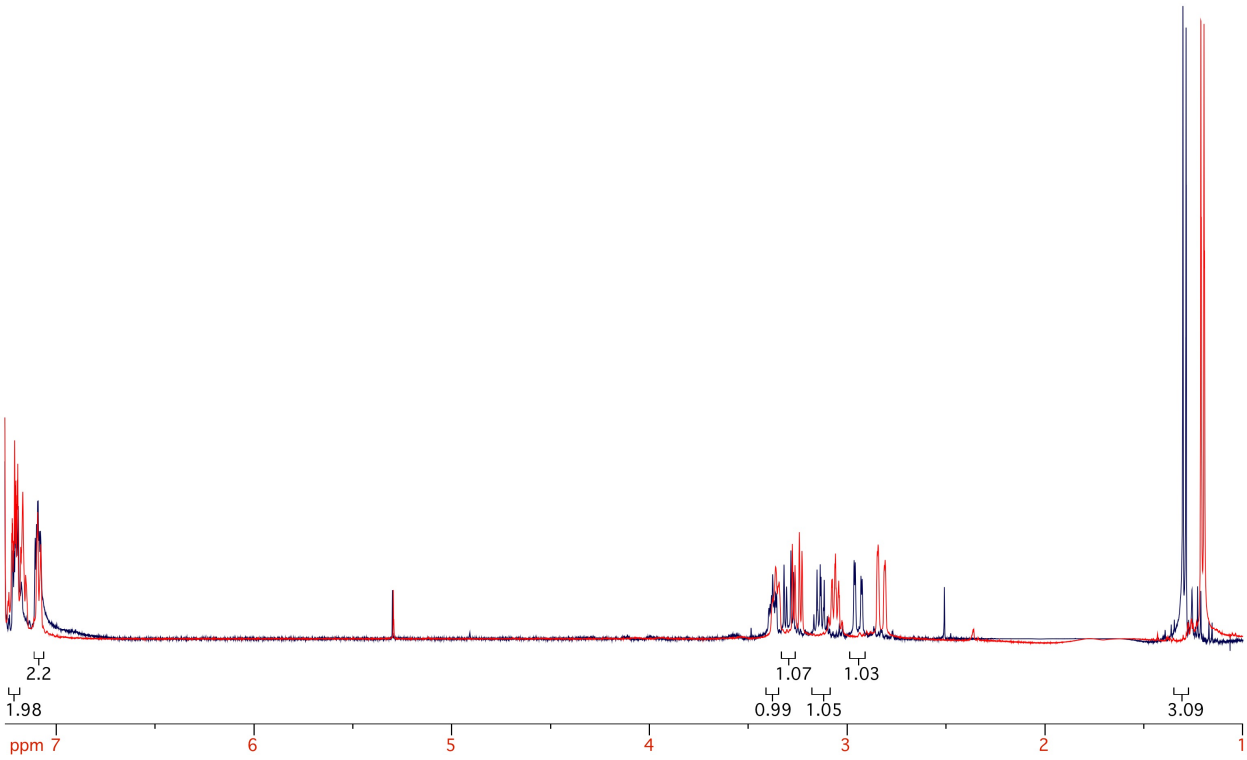
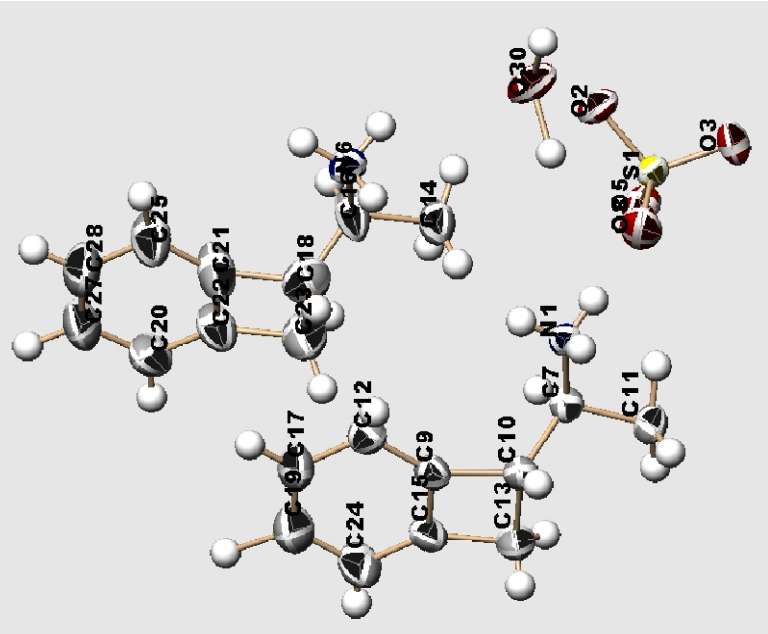


Figure 8.5. X-ray crystal structure for 8.15a enantiomers (RS/SR) that eluted from the column first



Each pair of enantiomers of **8.15a** was submitted for biological screening at dopamine and serotonin transporters and the results will be reported elsewhere. We hypothesize that the binding affinity of these compounds will be equal to or greater than their parent phenethylamine analogs.

8.4 Conclusion

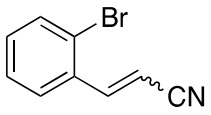
In summary, a synthetic route to aminobenzocyclobutene analogs of prototypical phenethylamines has been explored. The most straightforward route proceeded via [2 + 2] cycloaddition reaction with the diazonium salt of anthranilic acid to key intermediate 1-cyanobenzocyclobutene. The nitrile was methylated using a Grignard-mediated process

with the resulting imine directly reduced to the corresponding amine, affording the desired phenethylamine analog. Alternative methods were also explored with mixed success; several substituted anthranilic acids did not produce the desired 1-cyanobenzocyclobutene. Therefore, for products with additional substitution on the aromatic ring, an alternative process was recommended. From 2-bromohydrocinnamonitriles, the benzocyclobutene core was synthesized with the added advantage of regioselectivity. The work presented here represents an ongoing effort and biological screening of target compounds is currently underway and will dictate future motivation. Further exploration and optimization of synthetic methods presented here is likely.

8.5 Acknowledgement

This research was funded by the University of New Orleans College of Sciences Graduate Research Grant and the University of New Orleans.

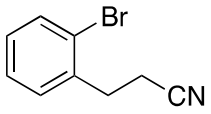
8.6 Experimental Section



3-(2-bromophenyl)acrylonitrile (8.11a)

A dry 3-neck round bottom flask was charged with 40 mL anhydrous acetonitrile and 85% KOH (5.25 g, 80.0 mmol: based on 85% purity). A condenser was attached and a nitrogen atmosphere was established. The suspension was brought to reflux and 1-bromobenzaldehyde (9.25 g, 50.0 mmol) dissolved in dry acetonitrile (20 mL) was added

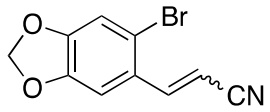
via syringe over a 30 second period. The reaction was allowed to proceed for an additional 60 seconds then immediately poured over 100 g of crushed ice. The resulting aqueous suspension was extracted with methylene chloride (3 x 100 mL). Combined organic extracts were washed with brine then dried over Na_2SO_4. Removal of volatile materials under reduced pressure on a rotary evaporator yielded a brown-purple oil which was purified by CombiFlash using 10% EtOAc in hexanes isocratic. Fractions collected proved to be **8.11a** (4.2 g, 42%) as a shiny white solid, mp 55-56 °C. The product was a 4.5 : 1 mixture of E:Z isomers, determined by ^{1}H-NMR peak integration. (E-isomer) ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.03 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.65 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.51 (d, *J* = 12.0 Hz, 1H), 7.43 (td, *J* = 7.6, 1.0 Hz, 1H), 7.30 (td, *J* = 7.7, 1.6 Hz, 1H), 5.60 (d, *J* = 12.0 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 149.3, 133.9, 132.4, 129.8, 128.2, 127.2, 125.0, 117.9, 99.3



3-(2-bromophenyl)propanenitrile (8.9a)

To a dry 100 mL round bottom flask was added anhydrous pyridine (28 mL) and anhydrous methanol (9 mL) then, with stirring, **8.11a** (3.8 g, 18 mmol) was dissolved. $NaBH_4$ granules (0.9 g, 24 mmol) were then added over 1 minute. Some gas evolution was observed as the light yellow solution turned orange-red. A condenser with drying tube attached was fixed to the flask and a gentle reflux was established for 2 h. Upon cooling to room temperature, the reaction mixture was dumped over 100 mL HCl (10%) containing ice. Additional HCl (6N) was added dropwise until with mixture appeared acidic on pH paper. The pink solution was extracted with diethyl ether (3 x 75 mL). Combined organic

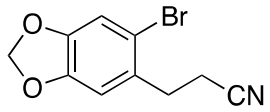
extracts were washed with 10 % HCl (3 x 15 mL) until pink color, suspected to be pyridine, disappeared followed by water and brine then dried over Na_2SO_4. The extracts were concentrated to **8.9a** (3.6 g, 94 %) as a light yellow oil of adequate purity for further synthesis. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.57 (d, *J* = 7.8 Hz, 1H), 7.33-7.28 (m, 2H), 7.19-7.13 (m, 1H), 3.10 (t, *J* = 7.4 Hz, 2H), 2.69 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 137.4, 133.4, 131.0, 129.4, 128.2, 124.3, 119.1, 32.3, 17.8



3-(6-bromobenzo[*d*][1,3]dioxol-5-yl)acrylonitrile (8.11b)

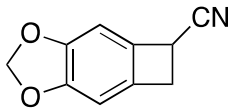
A dry 3-neck round bottom flask was charged with anhydrous acetonitrile (20 mL) and freshly powdered KOH (1.0 g, 16 mmol, based on 85 % purity). A condenser was attached and a nitrogen atmosphere was established. The suspension was brought to reflux and 1-bromopiperonal (2.3 g, 10 mmol) dissolved in 40 mL dry acetonitrile was added via syringe over a 60 second period. The reaction was allowed to proceed for an additional five minutes at reflux then immediately poured over 100 g of crushed ice. The resulting aqueous suspension was extracted with methylene chloride (3 x 40 mL). Combined organic extracts were washed with brine then dried over Na_2SO_4. Removal of volatile materials under reduced pressure on a rotary evaporator yielded a brown fecal looking paste that was purified by CombiFlash (15 % EtOAc in hexanes isocratic). Fractions collected proved to be **8.11b** (1.9 g, 75%) of a fine yellow powder, mp 168-169 °C. A small amount of unreacted starting material eluted before the product and can be recovered if desired. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.72 (d, *J* = 16.5 Hz, 1H), 7.06 (s, 1H), 6.96 (s, 1H), 6.04 (s, 2H),

5.67 (d, *J* = 16.5 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 151.1, 148.9, 148.4, 126.9, 118.2, 117.9, 113.5, 105.7, 102.8, 96.8.



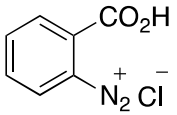
3-(6-bromobenzo[*d*][1,3]dioxol-5-yl)propanenitrile (8.9b)

To a dry 50 mL round bottom flask was added anhydrous pyridine (9 mL) and anhydrous methanol (3 mL) then, with stirring, **8.11b** (1.5 g, 6.0 mmol) was dissolved. $NaBH_4$ granules (0.30 g, 7.5 mmol) were then added over 1 minute. Some gas evolution was observed as the light yellow solution turned orange-red. A condenser with drying tube attached was fixed to the flask and a gentle reflux was established for 2 h. Upon cooling to room temperature, the reaction mixture was dumped over 30 mL HCl (10%) containing ice. Additional HCl (6N) was added dropwise until with mixture appeared slightly acidic on pH paper. The pink solution was extracted with diethyl ether (3 x 30 mL). Combined organic extracts were washed with HCl (10 %) until pink color, suspected to be pyridine, disappeared followed by brine then dried over Na_2SO_4. The extracts were concentrated to **8.9b** (1.3 g, 83%) as a grape-colored oil that solidified upon sitting overnight, mp 77-78 °C. The tan solid was of adequate purity for further synthesis. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.01 (s, 1H), 6.78 (s, 1H), 5.98 (s, 2H), 2.99 (t, *J* = 7.3 Hz, 2H), 2.63 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 148.05, 147.87, 130.3, 119.1, 114.5, 113.2, 110.6, 102.2, 32.2, 18.0.



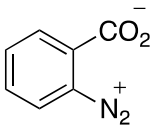
5,6-dihydrocyclobuta[4,5]benzo[1,2-*d*][1,3]dioxole-5-carbonitrile

A 100 mL 3-neck round bottom flask was charged with liquid ammonia (~60 mL) using a condenser containing dry ice and acetone. Sodium lump (0.25 g, 11 mmol) was cut into smaller sized chunks and with stirring carefully added to the ammonia, resulting in a deep blue colored solution. A pinch of $Fe(NO_3)_3 \cdot 9H_2O$ was added causing the blue color to slowly disappear over about 15 minutes resulting in a cloudy grey-brown suspension containing sodium amide. **8.9b** (0.5 g, 2 mmol) dissolved in a minimum amount of anhydrous THF was then introduced slowly. The reaction was allowed to stir for 4h in a dry ice acetone bath. Residual $NaNH_2$ was decomposed by careful addition of NH_4Cl (~1 g) in small portions. External cooling was removed and the ammonia was allowed to evaporate overnight. The brown residue was partitioned between H_2O (50 mL) and Et_2O (50 mL). The two-phase mixture with suspended solids was filtered through a celite pad and washed with additional Et_2O. The organic layer was collected and the aqueous was extracted with more with Et_2O (2 x 50 mL). Combined ether extracts were washed with water, brine and dried over Na_2SO_4 then concentrated under reduced pressure to a brown oil. Flash column chromatography (SiO_2, 0-10% EtOAc in hexanes) afforded **8.5b** (150 mg, 44%) as a tan oil. TLC: R_f 0.7 (30% EtOAc in hexanes). ^1H NMR (400 MHz; $CDCl_3$): δ 6.72 (s, 1H), 6.64 (s, 1H), 5.92 (s, 2H), 4.09 (dd, *J* = 5.1, 2.4 Hz, 1H), 3.52 (dd, *J* = 13.7, 5.3 Hz, 1H), 3.38 (dd, *J* = 13.7, 2.5 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 149.1, 148.0, 135.4, 130.6, 120.0, 105.4, 104.6, 100.8, 35.1, 27.7.



Benzodiazonium 2-carboxylate hydrochloride (Route I, 8.12a)

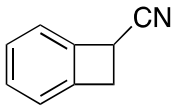
A solution of anthranillic acid (2.74 g, 20.0 mmol) in 30 mL of absolute ethanol was cooled in an ice bath and treated with 2 mL of concentrated hydrochloric acid. Cold isoamyl nitrite (5.0 mL, 38 mmol) was added dropwise to the stirred solution at 0°C over approximately 10 minutes. The color of the solution became deep orange initially then became lighter towards the end of the addition. Approximately 30 mL of diethyl ether was added causing the precipitation of the diazonium salt, which was collected and washed with additional ice cold ether until the washings were colorless affording **8.12a** (3.0 – 3.4 g wet) as a white solid. The compound was used immediately without further purification of characterization.



Benzodiazonium 2-carboxylate (Route II, 8.12b)[14]

A solution of anthranillic acid (2.74 g, 20.0 mmol) and trifluoroacetic acid (c. five drops) in tetrahydrofuran (30 mL) was prepared in a 100 mL beaker and cooled in an external ice bath. With stirring, isoamyl nitrite (5.0 mL, 38 mmol) was added over about 1 minute. A transient red solid appeared then slowly disappeared. The mixture continued stirring and was allowed to come to room temperature over 1 hour, then cooled to about 10°C. The resulting tan precipitate was carefully collected using vacuum filtration, ensuring that it never dried completely, and washed with cold tetrahydrofuran until the washings were

colorless affording **8.12b** (2.8 – 3.0 g wet) as a tan solid. The compound is explosive upon scraping and heating and must be used immediately, consequently, no further characterization was carried out.



bicyclo[4.2.0]octa-1,3,5-triene-7-carbonitrile (8.5a)[13]

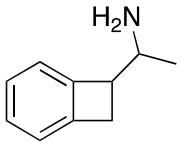
Method A, from 8.9a: A 250 mL 3-neck round bottom flask was charged with ~100 mL liquid ammonia using a condenser containing dry ice and acetone. Sodium lump (1.3 g, 55 mmol) was cut into smaller size chunks and with stirring carefully added to the ammonia, resulting in a deep blue colored solution. A pinch of $Fe(NO_3)_3 \cdot 9H_2O$ was added causing the blue color to slowly disappear over about 15 minutes resulting in a cloudy grey-brown suspension containing sodium amide. **8.9a** (2.1 g, 10 mmol) dissolved in a minimum amount of anhydrous THF was then introduced slowly. The reaction was allowed to stir for 4h in a dry ice acetone bath. Residual $NaNH_2$ was decomposed by careful addition of NH_4Cl (~2-3 g) in small portions. External cooling was removed and the ammonia was allowed to evaporate overnight. The brown residue was partitioned between H_2O (100 mL) and Et_2O (100 mL). The two-phase mixture with suspended solids was filtered through a celite pad and washed with additional Et_2O. The organic layer was collected and the aqueous was extracted with more with Et_2O (2x 100 mL). Combined ether extracts were washed with water then dried with brine and dried over Na_2SO_4 then concentrated under reduced pressure to a brown oil. Flash column chromatography on (SiO_2, 0-10% EtOAc in hexanes) afforded **8.5a** (0.4g, 31%) as a light yellow oil. TLC: R_f 0.7 (30% EtOAc in hexanes). ^1H NMR

(400 MHz; $CDCl_3$): δ 7.36-7.29 (m, 2H), 7.23 (d, *J* = 7.1 Hz, 1H), 7.14 (d, *J* = 6.5 Hz, 1H), 4.26 (dd, *J* = 5.5, 2.8 Hz, 1H), 3.70 (dd, *J* = 14.2, 5.6 Hz, 1H), 3.56 (dd, *J* = 14.1, 2.8 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 143.3, 139.2, 129.7, 128.5, 123.7, 122.8, 119.9, 36.5, 29.1.

Method B, from 8.12a: Wet diazonium chloride **8.12a** (3 - 3.5 g, ~20 mmol) was carefully transferred to a reaction flask and suspended in toluene (50 mL) and acrylonitrile (30 mL). Propylene oxide (2.1 mL, 30 mmol) was introduced and a balloon was stretched over the neck of the flask and the reaction was maintained at 40°C overnight. Collection of about 0.5L of gas signaled reaction completion. Volatile materials were removed under reduced pressure on a rotary evaporator and the residue was subjected to flash column chromatography (SiO_2 0-10% EtOAc in hexanes) to afford **8.9a** (1.7 g, 66%) as a light yellow oil. The product eluted as a light yellow band just ahead of an intense blue band on the column. Characterization proved to be identical to the material obtained using **Method A.**

Method C, from 8.12b: Wet diazonium carboxylate **8.12b** (2.8 - 3 g, ~20 mmol) was carefully transferred to a reaction flask and suspended in dichloroethane (15 mL). Acrylonitrile (1.5 g, 28 mmol) was added to the flask and the mixture was gently heated and swirled over a hotplate until gas evolution ceased (about 1 hour). Volatile materials were removed under reduced pressure on a rotary evaporator and the residue was subjected to flash column chromatography (SiO_2, 0-10% EtOAc in hexanes) to afford **8.9a** (1.0 g, 38%) as a light yellow oil. The product eluted as a broad light yellow band just

behind a tight intense yellow band on the column. Characterization proved to be identical to the material obtained using **Method A.**



1-(bicyclo[4.2.0]octa-1,3,5-trien-7-yl)ethanamine (8.15a)

A 1M solution of methylmagnesium iodide was prepared by slowly introducing methyl iodide (3.4 g, 24 mmol) in 24 mL Et_2O to Mg turnings (0.58 g, 24 mmol) under dry inert conditions and stirring until complete disappearance of Mg occurred, approximately 30 minutes. Using a pressure-equalizing addition funnel, 5 mL of the Grignard solution (~5 mmol MeMgI) was introduced over 30 minutes to a stirring solution of **8.9a** (0.65 g, 5 mmol) in THF (20 mL) on ice, resulting in a banana-colored suspension. The ice bath was removed and the reaction was allowed to stir at room temperature for 2 h.

Imine reduction: The reaction mixture was cooled to 0°C using an external ice bath and hopefully anhydrous methanol (15 mL) was added followed by $NaBH_4$ (473 mg, 12.5 mmol) and was allowed to stir at room temperature overnight. The resulting suspension was cooled on an ice bath and concentrated HCl was added until the mixture appeared slightly acidic on pH paper and was taken up in H_2O (50 mL). Additional concentrated HCl was required to ensure that the solution was slightly acidic. The aqueous extract was washed with three volumes of toluene then made alkaline with NaOH (4N) and was extracted with toluene (3 x 50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated to 0.5 g of yellow residue. Diastereomers were carefully resolved using

flash column chromatography (SiO_2, 2% MeOH, 0.5% NH_4OH, DCM). The diastereomers that eluted from the column first provided **(RS/SR)8.15a** (100 mg, 27%*) as a transparent oil followed by **(RR/SS)8.15a** (80 mg, 22%*) as a transparent oil. The sulfate salts of each were prepared by dissolving the freebases in 1-2 mL anhydrous isopropanol in a centrifuge tube and adding concentrated sulfuric acid (~30 mg). The tubes were centrifuged and the precipitate was isolated. The sulfate salts may be recrystallized from 10% water in ethanol with approximately 60% recovery. **(RS/SR)8.15a**: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.20-7.16 (m, 2H), 7.12-7.05 (m, 2H), 3.38 (ddd, *J* = 7.8, 5.4, 2.4 Hz, 1H), 3.26 (dd, *J* = 14.1, 5.3 Hz, 1H), 3.10-3.06 (m, 1H), 2.86-2.82 (m, 1H), 1.21 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 144.3, 143.1, 127.8, 127.0, 123.2, 122.6, 51.4, 50.7, 33.4, 21.7. **(RR/SS)8.15a**: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.22-7.19 (m, 2H), 7.11-7.07 (m, 2H), 3.38 (ddd, *J* = 7.9, 5.3, 2.5 Hz, 1H), 3.30 (dd, *J* = 14.0, 5.2 Hz, 1H), 3.13 (dq, *J* = 8.1, 6.4 Hz, 1H), 2.94 (dd, *J* = 13.9, 2.4 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): δ 145.9, 144.1, 128.0, 127.1, 123.3, 122.7, 51.0, 50.2, 33.9, 20.5.

*yields calculated on the assumption that starting material **8.9a** was an equimolar mixture of R and S enantiomers.

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VITA

Alexander Sherwood was born on July 20th 1987 to Herbert Sherwood and Monica Sherwood in Chalmette, Louisiana. Growing up in southern Louisiana, Alex pursued many hobbies and activities, including constructing and flying remote controlled aircraft, as well as various electronic and musical endeavors. Alex graduated from Andrew Jackson High School in 2005 with honors and subsequently attended The University of New Orleans on a full scholarship for exactly two weeks prior to Hurricane Katrina. He washed ashore in Baton Rouge and immediately continued his undergraduate studies at Louisiana State University with a general focus in science and engineering. Alex eventually developed a strong interest in neuroscience and pharmacology and saw chemistry as an avenue to pursue these interests. He moved back home to New Orleans in 2007 to complete a Bachelors degree in Chemistry at the University of New Orleans. During this time, he worked part-time in the Department of Neuroscience at Louisiana State University Health Sciences Center under the direction of Dr. Song Hong. Alex met his future graduate advisor Dr. Mark Trudell during his final year of undergraduate study in 2009. Alex expressed interests in conducting research in cannabinoid chemistry that ultimately led to the studies discussed in this dissertation. In 2013, Alex accepted a post-doctoral research fellowship with Dr. Thomas Prisinzano at The University of Kansas to continue studying the pharmacology of CNS-active molecules.